

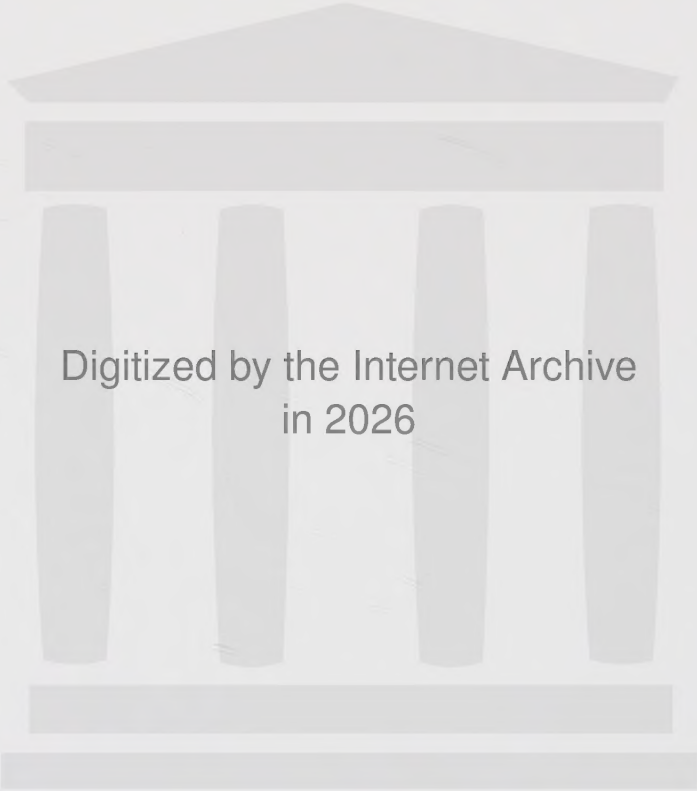
Metabolic Regulation of Lipoprotein Lipase and Hepatic Lipase

With special reference to the influence of thyroid hormones
and local innervation

PER HANSSON



LUND 1984



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Metabolic Regulation of Lipoprotein Lipase
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With special reference to the influence of
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Akademisk avhandling

av

Per Hansson

leg läkare, Ssk

Offentlig disputation för medicine doktorsgraden äger rum,
med vederbörligt tillstånd av medicinska fakulteten vid
Lunds Universitet, onsdagen den 6 juni 1984 kl 09.15 i
föreläsningssal 1, centralblocket, Lunds Lasarett.

Lund 1984

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION	
	Date of issue June 6, 1984	
	CODEN: LUMEDW/(MECL-1003)/1-67/(1984)	
Author(s) Per Hansson	Sponsoring organization	
Title and subtitle METABOLIC REGULATION OF LIPOPROTEIN LIPASE AND HEPATIC LIPASE With special reference to the influence of thyroid hormones and local innervation.		
Abstract The influence of thyroid hormones and local innervation upon the regulation of lipoprotein lipase(LPL) and hepatic lipase(HL)has been investigated. Hypothyroid patients have low activities of both enzymes in postheparin plasma,while hyperthyroid individuals display an elevation of HL activity,with that of LPL remain- ing unchanged.The same pattern is seen in volunteers given an excess of triiodothyronine Hypothyroid rats show a " paradoxical" increase in LPL activity of fatwhich seems due to an extracellular accumulation of enzyme.HL activity in liver is decreased.Triiodo- thyronine-treated animals display decreases inLPL activities of fat and heart while the HL activity increases.Triiodothyronine also augments HL secretion from cultured rat hepatocytes.The concomitant changes of food intake by dysthyroid rats leads to a modified response in enzyme activies,most pronounced for adipose tissue LPL. Microsurgical denervation of rat epididymal fat does not lead to altered tissue activity of LPL.Periportal denervation of rat liver is followed by a 20% increase in liver activity of HL.		
Key words Adipose tissue-hepatic lipase-lipoprotein lipase-liver,innervation-thyroid hormones		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN 91-7222-751-6
Recipient's notes	Number of pages 67	Price
	Security classification	

Distribution by (name and address) Per Hansson,Inst f klinisk kemi,Lasarettet
 S-221 85 Lund

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Date June 6, 1984

From the Department of Clinical Chemistry,
University of Lund, Lund, Sweden

Metabolic Regulation of Lipoprotein Lipase and Hepatic Lipase

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---zombies are the living dead: innocent victims raised in a comatose state-----and forced to toil indefinitely as slaves.

Davis 1983

This thesis is based on the following papers, which will be referred to by their Roman numerals:

- I Valdemarsson S, Hansson P, Hedner P, Nilsson-Ehle P, 1983: Relationship between thyroid function, hepatic and lipoprotein lipase activities, and plasma lipoprotein concentrations. Acta Endocrinol. 104:50-56.
- II Hansson P, Valdemarsson S, Nilsson-Ehle P, 1983: Experimental hyperthyroidism in man: effects on plasma lipoproteins, lipoprotein lipase and hepatic lipase. Horm. Metabol. Res. 15:449-452.
- III Hansson P, Nordin G, Nilsson-Ehle P, 1983: Influence of nutritional state on lipoprotein lipase activities in the hypothyroid rat. Biochim. Biophys. Acta 753:364-371.
- IV Hansson P, Valdemarsson S, Nilsson-Ehle P: Hepatic lipase and lipoprotein lipase activities in hypo- and hyperthyroid rats during free and restricted food intake. Submitted for publication.
- V Hansson P, Jensen E, Florén C-H, Nilsson-Ehle P: Thyroid hormones stimulate the release of hepatic lipase from cultured rat hepatocytes. Submitted for publication.
- VI Hansson P, Holmin T, Nilsson-Ehle P, 1981: Microsurgical denervation of rat adipose tissue: lack of effect on lipoprotein lipase. Biochem. Biophys. Res. Comm. 103:1254-1257.
- VII Hansson P, Lindfeldt J, Ekelund M, Kullendorff C-M, Holmin T, Nilsson-Ehle P: Hepatic lipase activity increases after liver denervation in the rat. Submitted for publication.

ABBREVIATIONS

ACTH = adrenocorticotrophic hormone

apo = apolipoprotein

C = cholesterol

CE = cholesteryl ester

cAMP = cyclic 3',5'-adenosine monophosphate

chylo = chylomicron/s/

HL = hepatic lipase

HDL = high density lipoprotein

IDL = intermediate density lipoprotein

ITU = 5-iodo-2-thiouracil

LCAT = lecithin: cholesterol acyl transferase

LDL = low density lipoprotein

LPL = lipoprotein lipase

mU = milli-unit of enzyme activity (1 nmol fatty acid released per minute at 37°C)

PL = phospholipids

PTU = 6-propyl-2-thiouracil

rT₃ = 3,3',5'-triiodothyronine, reverse T₃

SDS = sodium dodecyl sulfate

T₃ = 3,5,3'-triiodothyronine

T₄ = 3,5,3',5'-tetraiodothyronine, thyroxine

TG = triglycerides

TSH = thyroid-stimulating hormone, thyrotropin

VLDL = very low density lipoprotein

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General Introduction

Energy demands of the mammalian organisms are filled mainly by intake of fats (lipids) and sugar (carbohydrates). Although in our society daily requirements can be met by corresponding energy intake, we carry energy depots, mainly as fat, which could theoretically maintain us for 2-3 months. Several transport systems interact to provide a delicately integrated pattern of energy supply from the gut and from energy depots to the various organs of the body; the energy fluxes are dynamically changing to fill the specific demands posed by different metabolic situations such as physical activity, stress, pregnancy, and lactation. Carbohydrate energy is freely transported dissolved in the blood plasma, but the water-insoluble lipids require a specialized transport system, the plasma lipoproteins. Enzymatic degradation is a prerequisite for the uptake of lipid from these particles into the tissues. Two enzymes, lipoprotein lipase (LPL), and hepatic lipase (HL) occupy key positions in this process. The activities of these enzymes are major points of regulation in lipid transport and subject to complex hormonal, and possibly nervous, regulation.

The integration of energy transport may be disturbed as a consequence of disease, or illness may result from the lack of precise transport regulation. For example, renal and liver disease are almost always accompanied by secondary changes in lipid and carbohydrate metabolism; diabetes, obesity and hereditary defects in lipid transport represent primary disorders of energy transport. Frequently, the major clinical symptoms are not directly related to the disturbance in energy metabolism; instead, the impact of alterations in LPL and HL activities on the concentrations and composition of plasma lipoproteins may be expressed as for instance an accelerated development of atherosclerotic cardiovascular disease.

This thesis is part of a long-term project regarding the regulation of lipid transport and lipoprotein metabolism; it is specifically concerned with the regulation of LPL and HL by thyroid hormones and nervous activity. The influence of thyroid function on these enzymes has so far not been studied in great detail, although thyroid disease is comparatively common and is associated with profound changes in lipid transport. The concept of nervous regulation is generally recognized both for adipose tissue and liver. How-

ever, the lack of suitable experimental techniques has hampered direct studies on the neural regulation of enzyme activities.

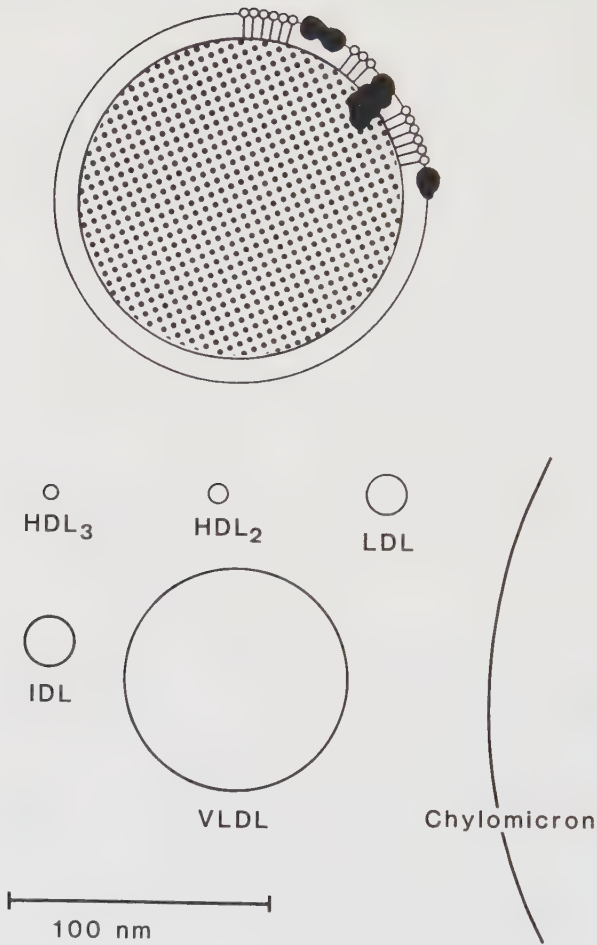


Fig 1. Schematic structure (top) and approximate relative sizes of human plasma lipoproteins. The surface film is composed mainly of phospholipids and unesterified cholesterol. Different apolipoproteins are also integrated into the surface of the particles (black symbols). The core of chylomicrons and VLDL is an amorphous mixture of triglyceride and esterified cholesterol, while the degree of organization in the core of small particles (LDL, HDL) is higher.

Lipoprotein lipase

Lipoprotein lipase (LPL) was first discovered as a factor involved in the clearing of opalescent, triglyceride-rich dog plasma following intravenous administration of heparin (Hahn 1943). The term "lipoprotein lipase" was coined by Korn (1954, 1955), who also established criteria for identification of LPL activity: a) activation by a serum co-factor, b) alkaline pH-optimum and c) inhibition by 1M NaCl and by protamine.

Molecular characteristics

LPL has been purified from several sources, including postheparin plasma, adipose tissue, heart muscle, and bovine skim milk (Nilsson-Ehle et al. 1980). The molecular weight varies between different preparations (Östlund-Lindqvist 1978, Smith & Pownall, in press); SDS-polyacrylamide gel electrophoresis gives values of 50-70,000 (cf, however, Fielding et al. 1977). In contrast, values of 100-130,000 are reported in studies using non-dissociating techniques, such as ultracentrifugation (Iverius & Östlund-Lindqvist 1976) and radiation inactivation (Garfinkel et al 1983). It thus appears that the physiologically active enzyme is a dimer. Its primary structure is not known; the amino acid composition is similar in LPL from different species (Nilsson-Ehle et al 1980). Several studies indicate that the active site comprises histidyl and seryl residues (Vainio et al 1982, Quinn et al 1982, Kinnunen et al 1983). LPL is a glycoprotein (Iverius & Östlund-Lindqvist 1976); the carbohydrate moiety is apparently needed for secretion of the enzyme (Cryer 1981). Fielding and co-workers (1977) have demonstrated the presence of LPL isoenzymes in heart and adipose tissue. The isoenzymes differ both in molecular weight and in the kinetic characteristics (see below).

Enzymological features

In vitro, LPL catalyzes the hydrolysis of a variety of triglycerides as well as diglycerides, monoglycerides, phospholipids and synthetic substrates such as Tween 20 and p-nitrophenyl esters (Egelrud & Olivecrona 1973, Chung & Scanu 1977, Fielding et al. 1977). In mixtures of different glyceride substrates, monoglycerides are preferentially hydrolyzed (Twu et al. 1976). The enzyme attacks preferentially the sn-1, but also the sn-3

bonds; there is not activity against secondary ester bonds (Nilsson-Ehle 1974, Smith & Pownall, in press).

Several activators of LPL have been described. The most important one is apolipoprotein CII (apo CII), which represents the serum factor originally described by Korn (1955). Its structure and function have been extensively reviewed recently (Quinn et al 1982, Smith & Pownall in press). The apolipoprotein contains separate sequences which bind phospholipid, and bind and activate LPL. The presence of apo CII is necessary for LPL activity; this is highlighted by the genetic disorder, apo CII-deficiency. In this condition, functional LPL activity is zero, though the enzyme may be activated by exogenous apoprotein (Breckenridge et al 1978). Apolipoprotein H (β_2 -glykoprotein I) has been reported to enhance LPL activation by apo CII (Nakaya et al 1980). The effect of heparin is mainly to release the enzyme from the tissue (see below); it also stabilizes LPL but apparently does not activate the purified enzyme (Jackson et al. 1980, Quinn et al. 1982). However, Staprans et al. (1980, 1981) have isolated an LPL activator, which appears to be heparan sulphate. The effect of heparin is not related to its anti-coagulant properties (Bengtsson et al. 1977, 1980, Persson et al. submitted).

Several substances inhibit the LPL reaction. Some of the inhibitors are experimental tools, whereas others may have a physiological function. The virtually complete inhibition in vitro by 1M NaCl is one of the primary criteria for LPL. Though the exact mechanism is not clear, an interaction of the chloride ions with the LPL - apo CII - complex has been proposed. (Fielding & Fielding 1976). Protamine also inhibits the enzyme, but even at very high concentrations of inhibitor there is still a residual activity (Twu et al. 1975). Immunoinhibition of LPL via specific anti-LPL-immunoglobulins is a powerful experimental tool (Kompiang et al. 1976).

Endogenous lipoprotein components have been implicated as physiological LPL inhibitors. The product inhibition exhibited by fatty acids and monoglycerides in vitro is well established; it has been proposed that such a mechanism is operative also in vivo (Bengtsson & Olivecrona 1980a). LPL inhibition by unesterified cholesterol and sphingomyelin has been implicated in the pathogenesis of dyslipoproteinemia (Fielding 1970, Nilsson-Ehle et al.

1980, Quinn et al. 1982). Apo AI, apo CI, and apo E all reverse the apo CII-mediated activation in vitro (Ekman & Nilsson-Ehle 1975). The physiological significance of these findings is, however, still unclear.

LPL turnover

LPL is a secretory enzyme which is synthesized in the parenchymal cells of various organs. Its turnover has been most extensively studied in adipose and muscle tissues, but the general pattern is considered valid also for other organs (Fig 2). The enzyme undergoes post-translational modification, including glycosylation and possibly deletion of part of the peptide chain (Robinson & Wing 1970, Ashby et al 1978). By mechanisms involving the microtubular system, the parent cells actively secrete the enzyme (Nilsson-Ehle 1982). It is unclear if secretion also is associated with activation, which could be conceived in light of the different characteristics of intra- and extracellular forms of LPL (Nilsson-Ehle et al. 1976).

Largely by unknown mechanisms, the enzyme transported through the interstitium to the luminal surface of the endothelium, where it binds to the glycosaminoglycan, heparan sulphate, possibly in complex with the structural protein, fibronectin (Cryer 1983). It is now firmly established that endothelial cells do not synthesize LPL themselves, but avidly bind pre-formed enzyme (Björntorp et al. 1983). The secreted enzyme normally has a half-life of a few hours (Nilsson-Ehle et al. 1980). Little is known about the ultimate fate of the enzyme; after intravenous injection of ^{125}J -labelled LPL to rats, radioactivity is cleared from the blood to the liver within minutes (Wallinder et al. 1979). It has been proposed that LPL may be swept away from the tissues by its lipoprotein substrate and then be transported to the liver where it might function as a signal for hepatic uptake of lipoprotein remnants (Felts et al. 1975).

Regulation of the LPL reaction

The LPL reaction is the rate-limiting step in transport of lipid energy from the bloodstream to tissues. It is subject to extensive regulation which occurs by passive and active mechanisms. (Nilsson-Ehle et al. 1980, Cryer 1981, Nilsson-Ehle 1982).

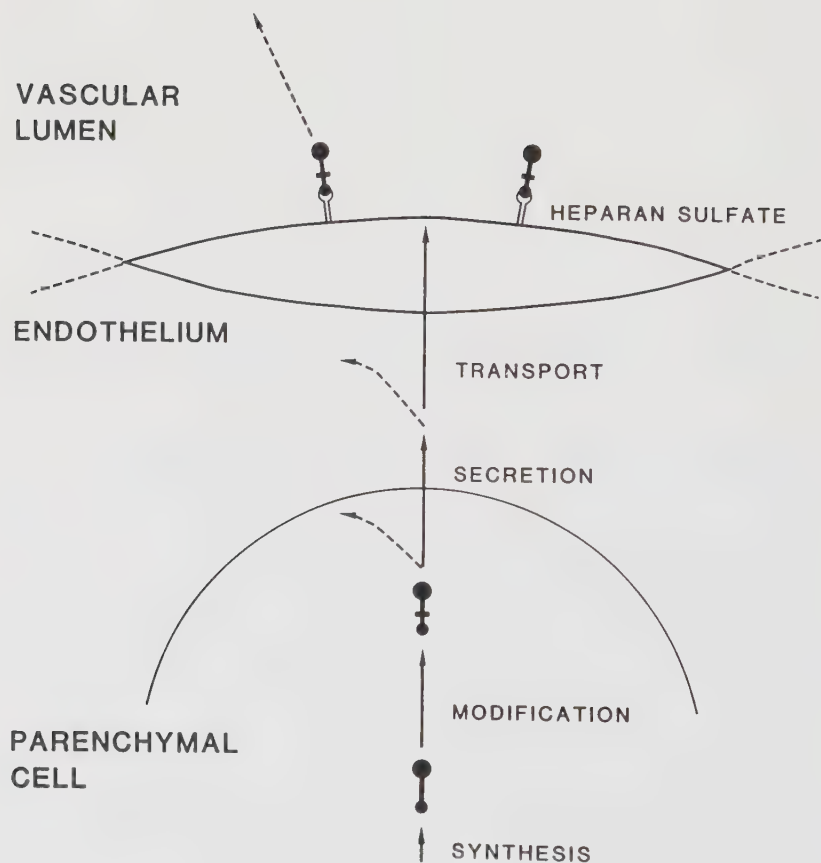


Fig 2. Schematic outline of LPL turn-over. For details see text. The dashed arrows represent possible sites of enzyme inactivation.

Since the LPL system does not seem to be saturated under physiological conditions the rate of the LPL reaction varies constantly with the amount of substrate present. If plasma triglyceride concentrations tend to rise (e.g. after eating), the excess will be eliminated from the circulation by means of a passive increase in the LPL reaction rate. Thereby, the plasma triglyceride pool is kept relatively constant, despite the high turnover of the substrate. The different localizations as well as kinetic properties of LPL isoenzymes provide the basis for a differential deposition of fat in various organs by passive mechanisms (Fielding & Havel 1977a). The LPL of rat heart has a low apparent K_m versus chylomicron triglyceride (0.07 mM); the enzyme reaction rate is thus almost maximal even at low plasma concentrations of chylomicrons. In contrast, adipose tissue LPL (apparent K_m 0.70 mM) is not saturated at the low substrate concentrations. Thus, the post-prandial increase in chylomicron input will increase the LPL reaction rate in adipose tissue, whereas lipid uptake in heart will hardly be affected, being already almost maximal. The physiological result is a preferential deposition of lipid in the adipose tissue after a meal.

Besides the passive LPL regulation, the enzyme is also regulated actively in response to various metabolic situations (see e.g. Cryer 1981). Differential changes in different tissues is common. For example, feeding stimulates the enzyme activity of adipose tissue, whereas that of heart is decreased. The reverse changes are seen during fasting. In lactating female rats, the LPL activity of the mammary gland is elevated, while it is depressed in adipose tissue. Hypophysectomy reverts these changes, but they are retained if the operated rats are given injections of prolactin (Zinder et al. 1974).

Active regulation provides a further means of ensuring an adequate flux of lipid energy to relevant tissues for oxidation, storage or secretion. In some cases, these physiological alterations in enzyme activity have been attributed to specific hormonal effects. (Borensztajn 1979, Cryer 1981). In addition, drugs such as theophylline (Robinson & Wing 1970), nicotinic acid (Shafrir & Biale 1971) and ethanol (Nilsson-Ehle 1979) affect the enzyme. The same substance may have the opposite effects in two tissues, as is described above in the case of prolactin. The influence of thyroid hormones on LPL is rather complicated and is discussed in detail below. There have been speculations of nervous regulation of the enzyme, since it is well

known that catecholamines depress the enzyme activity in adipose tissue (Robinson & Wing 1970), and since adipose tissue is richly supplied with adrenergic nerves (Rosell & Belfrage 1979).

The active enzyme regulation is accomplished at different levels. The synthesis of LPL is enhanced by several hormones, such as insulin (Spooner et al. 1979) and corticosteroids (Ashby & Robinson 1980). Posttranslational modification seems to be obligatory for LPL secretion, but is considered less likely to be involved in its regulation (Ashby et al. 1978). Secretion of the enzyme is known to be regulated by insulin (Garfinkel et al. 1976, Spooner et al. 1979) and, probably, by gastrointestinal hormones (Bourdeaux et al. 1980). A substantial amount of LPL may be inactivated before secretion from adipocytes. The inhibitory effect of catecholamines and cAMP may involve this mechanism (Ashby et al. 1978, Ashby & Robinson 1980). Cryer (1981) has proposed that once secreted from its parent cell, the enzyme is not subject to regulation. However, this notion may be challenged by the findings of other experiments. Inhibition of protein synthesis (by actinomycin D or cycloheximide) somewhat paradoxically increases LPL activity in whole adipose tissue, while the enzyme activity of cultured adipocytes is depressed by the same substances (Robinson & Wing 1970, Ashby et al. 1978, Spooner et al. 1979). These data may be explained by effects on an extracellular LPL-degrading system (Nilsson-Ehle 1982).

The LPL regulation in man and the rat differ in some aspects: for example, estrogens augment the activity of the human enzyme (Nikkilä et al. 1982). In contrast, LPL activity is decreased in the parametrial adipose tissue of estrogen-treated, ovariectomized rats. The concomitant decrease in feeding in these animals may contribute to the observed results (Ramirez 1981). Differences in the case of thyroid hormones will be discussed below.

Hepatic lipase

Hepatic lipase (HL) has been established as an enzyme, separate from LPL, only during the last decade (la Rosa et al. 1972, Augustin & Greten 1979, Kinnunen et al. 1983). HL, like LPL, is released into the circulation upon intravenous administration of heparin (la Rosa et al. 1972). However, HL is enzymatically distinct from LPL: full activity is retained in the presence of

1 M NaCl, (la Rosa et al. 1972) and the lipase activity is resistant to protamine (la Rosa et al. 1972, Krauss et al. 1974). Specific antibodies which do not cross-react with LPL have been raised (Huttunen et al. 1975). The enzyme has been less extensively studied than LPL, but has recently attracted considerable interest in view of its probable involvement in the metabolism of the "anti-atherogenic" HDL (Nikkilä et al. 1982).

For some years, HL was thought to originate exclusively from the liver. In 1980, however, Jansen and co-workers demonstrated the presence of HL in adrenals (1980, 1980a, Murase et al. 1982). Subsequently, the enzyme has also been found in ovarian tissue (Jansen & deGreef 1981). In view of its wider distribution, the lipase may hardly be referred to as "hepatic". For want of a better, generally accepted, name, I have retained "hepatic lipase" (HL) throughout this thesis.

Molecular characteristics

Several groups have purified HL from various sources (Assmann et al. 1973, Ehnholm et al. 1975, Östlund-Lindqvist 1978, Kuusi et al. 1979, Jensen & Bensadoun 1981, Frost et al. 1982, Twu et al. 1984). It is a glycoprotein, with an apparent molecular weight of about 50-60,000 (in SDS-polyacrylamide gel electrophoresis) or about 180-220,000 (by gel filtration). These data indicate that the functional enzyme is an oligomer. The amino acid composition of HL is distinct from that of LPL (Östlund-Lindqvist 1978); previous reports to the opposite effect have probably resulted from contamination of enzyme preparations with antithrombin III. The active site of HL may include seryl and histidyl residues (Kinnunen et al. 1983, Twu et al. 1984).

Enzymological features

Some of the characteristics of HL activity have already been mentioned. Its pH optimum is 9-9.5 (Assmann et al. 1973, Nilsson-Ehle & Ekman 1977). The substrate specificity is low: tri-, di- and monoglycerides are hydrolyzed in vitro, as are phospholipids. The enzyme does not show any preference for specific acyl groups; phosphatidyl ethanolamine is more readily attacked than phosphatidyl choline (Ehnholm et al. 1975). A single active site catalyzes mono- and triglyceride hydrolysis (Kinnunen et al. 1983, Twu et al. 1984). Collagenase treatment abolishes the activity against tri-

glycerides, without affecting monoglyceride hydrolysis (Kuusi et al. 1979a). In analogy with LPL, HL does not attack sn-2-bonds of glycerides (Åkesson et al. 1976); sn-1-bonds are more readily attacked than those in the sn-3 position (Kinnunen et al. 1983). The possible physiological substrates for HL will be discussed below.

Absence of serum in the enzyme assay does not result in decreased HL activity (Nilsson-Ehle & Ekman 1977), and no co-factor has been considered necessary for its physiological function. However, Jahn et al. (1981, 1983) have reported that apo A II specifically increases the rate of triglyceride hydrolysis by HL in vitro. These data contrast with those of Kubo et al. (1982), who report an inhibition by roughly the same concentration of this apolipoprotein, and attribute the effect to displacement of HL from substrate particles. It should be noted that different substrate emulgators were used in the two studies (phosphatidyl choline and gum arabic). The inhibitory properties of apo AI, CIII, and possibly CI, CII, are more generally accepted (Kinnunen & Ehnholm 1976, Kubo et al. 1982, Jahn et al. 1983, Twu et al. 1984). It has been proposed that SDS may selectively and quantitatively inhibit HL, while leaving LPL activity unchanged (Baginsky & Brown 1977). We have not been able to reproduce this finding.

Turnover of HL

Comparatively little is known about the turnover of the enzyme, but there are obvious similarities with LPL. HL is synthesized in the hepatocytes proper (Jansen et al. 1979); the cellular origin in ovaries and adrenals has not been determined. After glycosylation, HL is secreted from hepatocytes and is transported to the endothelium, presumably by the microtubular system (Chajek et al. 1977). Endothelial cells do not produce the enzyme, but avidly bind pre-formed HL (Jansen et al. 1980b) at glycosaminoglycan residues (Cryer 1983). Virtually all HL activity is located on the endothelium (Kuusi et al. 1979b). It has been proposed that HL is located in, or near, the coated pits containing apolipoprotein B/E receptor. The enzyme would then be interiorized together with remnant lipoproteins and inactivated by lysosomal enzymes (Chajek et al. 1977, Kinnunen et al. 1983).

Regulation of the HL reaction(s)

The physiological role of HL has not been clarified (see below). Nevertheless, the enzyme appears to be under metabolic control. Passive modulation of the reaction rate has not been described (cf LPL), but active, physiological control is well established. The enzyme activity is low in diabetic rats, but normalizes upon insulin substitution (Elkeles & Hambley 1977). Chronic insulin administration to normal rats increases the enzyme activity (Knauer et al. 1982). Hypothyroidism depresses HL release from perfused rat livers (Jubelin et al. 1978) and the enzyme activity is low in rat adrenals (Jansen & deGreef 1981) and post-heparin plasma of hypothyroid rats and man (Murase & Uchimura 1980, Krauss et al. 1974, Valdemarsson et al. 1982). Conversely, triiodothyronine treatment or hyperthyroidism increases the enzyme activity of rat adrenals (Jansen & deGreef 1981) and in human post-heparin plasma (Valdemarsson et al. in press); post-heparin plasma of thyroxine-treated rats, however, does not exhibit elevations in the activity of HL (Murase & Uchimura 1980). Considerable interest in the effects of sex hormones on HL originates from the discovery by Ehnholm et al. (1975a) that oxandrolone (a gestagen/anabolic steroid) increases the enzyme activity in post-heparin plasma. It is also well established that estrogens depress the activity (Glueck et al. 1976, Jubelin et al. 1979). The effective doses of these hormones are those relevant in contraception (Tikkanen et al. 1982).

The studies on the effects of ACTH are especially interesting, since they demonstrate a differential effect on the enzyme activities of liver and adrenals. Cortisol treatment depresses HL activity in both organs (Jansen & Hülsmann 1975, Jansen & de Greef 1981). In contrast, ACTH treatment clearly elevates the activity in adrenals, while that of the liver is still depressed (Jansen et al. 1983, Schoonderwoerd et al. 1983). These data strongly suggest that HL activity of adrenals is regulated by ACTH, while the liver enzyme seems to respond to cortisol. Based on these findings, Jansen & Hülsmann (1980a) have proposed that the adrenal enzyme is involved in the tissue uptake of cholesterol to provide substrate for steroid hormone production. This is supported by positive correlations between HL activities in ovaries of lactating rats and the serum concentrations of progesterone and 20- α -OH-progesterone (Jansen & deGreef 1981).

The molecular mechanisms of hormone action are still largely unknown. Schoonderwoerd et al. (1983) have shown that secretion of HL is diminished in cultured hepatocytes from cortisol-treated rats. Also, liver endothelial cells cultured from such animals have a decreased capacity to bind HL.

Table I. Lipoproteins of human plasma

	Density (kg/l)	Approx.size (nm)	Main Lipid	Main Apoprotein	T _{1/2}
Chylomicrons	<0.95	>75	TG	B-48	minutes
VLDL	0.95-1.006	25-75	TG	B-100	hours
IDL	1.006-1.019	22-24	TG,CE	B-48,B-100,E	minutes
LDL	1.019-1.063	20-23	CE	B-100	5-9 days
HDL ₂	1.063-1.125	6-14	PL,CE	AI,AII	5 days
HDL ₃	1.125-1.210	4-10	PL,CE	AI,AII	5 days

Plasma lipoproteins - a short overview

This chapter is, with necessity, condensed and the reader is referred to a number of excellent reviews (Nelson et al. 1972, Greten. 1976, Scanu et al. 1979, Lewis 1980, 1981, Carlson & Pernow 1982, Schonfeld 1983). It is important to understand that the lipoprotein fractions (Table I) are not static entities. On the contrary, continuous transfer and exchange of both lipid and protein moieties occur between different lipoprotein particles (Lewis 1980).

Molecular mechanisms in the transformation and degradation of lipoproteins
Three mechanisms cooperate in lipoprotein transformation: firstly, the lipid moieties are degraded and transformed by the action of enzymes, which are either anchored to the endothelium (LPL-HL) or associated with circulating lipoproteins (LCAT). Secondly, both lipid and protein rapidly equilibrate between different lipoprotein particles and cells, providing a means for exchange or net transfer of the components. The rate of exchange/transfer may be increased by the presence of specific carrier proteins. Thirdly, the elimination of lipoproteins from the plasma is modulated via several types of lipoprotein receptors (Mahley & Innerarity 1983). The delicate regulation of lipoprotein concentrations in plasma result from a complex interplay between regulatory mechanisms affecting enzyme activities and receptor numbers and activity. There are, so far, no data indicating a regulatory capacity of transfer reactions under physiological conditions.

Metabolism of triglyceride-rich lipoproteins in man

The triglyceride-rich chylomicrons (originating from the intestines) and VLDL (mainly from the liver) are secreted into the bloodstream as 'nascent' particles (Fig 3). Within the circulation, they are rapidly modified by the transfer of apolipoproteins, mainly from HDL (Havel et al. 1973). Most notably, they acquire apo CII, the obligatory co-factor for LPL. As a result, the lipoproteins become amenable to triglyceride hydrolysis by LPL. Delipidation of the core results in a successive increase in particle density. At the level of IDL, two metabolic pathways are possible: a direct uptake into the liver seems more common for chylomicron-derived particles (Redgrave 1970) while VLDL is mainly transformed into LDL (Faergeman 1977). Through the work of Brown, Goldstein et al. we know that LDL is taken up by peripheral tissues via a specific receptor for apo B, though

non-specific uptake ('the scavenger pathway') also occurs (Brown & Goldstein 1976, Goldstein & Brown 1982, 1982a). After hydrolysis of the cholesteryl esters, cholesterol may be used for membrane synthesis or production of other steroid compounds.

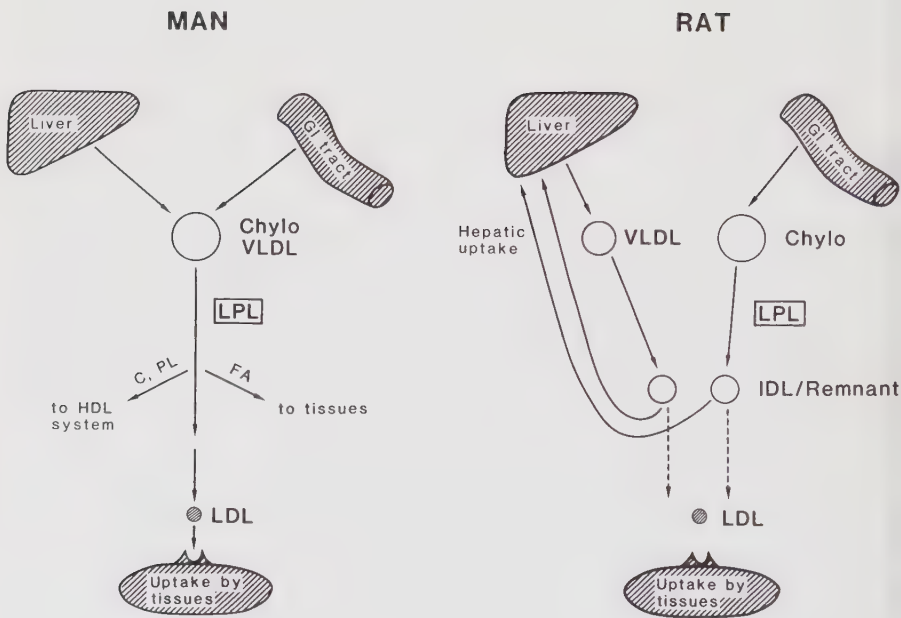


Fig 3. A schematic outline of the metabolism of triglyceride-rich lipoproteins in man and the rat. The LDL pathway is the dominating in our species; it constitutes only a minor route in the rat. As a result of LPL action a relative excess of unesterified cholesterol (C) and phospholipids (PL) will result; these compounds are transferred to the HDL system in both species.

FA, fatty acids

The HDL system and its role in the lipid metabolism of man

HDL particles are secreted by the liver and intestines; they may also be formed directly from chylomicrons (Lewis 1980). One of the major functions of this lipoprotein class is to act as acceptor of surface components (phospholipids, unesterified cholesterol and apoproteins). During the process of intravascular lipolysis, a relative excess of these compounds develops, which is transferred to HDL (Chajek & Eisenberg 1978, Eisenberg 1979). The surface-active phospholipids and cholesterol, once associated with HDL, are partly transformed by the HDL-associated enzyme, lecithin: cholesterol acyl transferase (LCAT), to form non-polar cholesteryl ester and water-soluble lyso-phospholipids. (Glomset 1979). Largely by this process, HDL₃ is transformed to HDL₂ (Patsch et al. 1978). Some of the cholesteryl esters formed are transferred back to the IDL-LDL fractions, a process facilitated by specific transfer proteins such as apo D (Chajek & Fielding 1978a).

Besides the role of HDL in intravascular lipoprotein turnover, it has also been proposed that HDL may extract cholesterol from tissues for transport to other organs, notably the liver; this pathway would provide a possibility to eliminate excess cholesterol from the organism (Glomset 1968, Norum et al. 1982). The theory, which has some experimental support (Fielding & Fielding 1983), could provide the basis for the anti-atherogenic potency of HDL (Connor 1979, Nikkilä et al. 1982).

Physiological roles of LPL and HL

The physiological role of LPL as the obligatory and rate-limiting factor in the triglyceride elimination from chylomicrons and VLDL is well established (Kompiang et al. 1976). In vitro, only this enzyme activity is needed (together with transfer proteins and HDL) for the formation of the "end product", LDL (Deckelbaum et al. 1979). Due to the LPL reactions, HDL₂ levels of plasma tend to increase, and LPL has been proposed as a major regulator of this lipoprotein fraction (Nikkilä et al. 1982). Only recently has it become evident that the presence of LPL is essential for the transmembrane transport of cholesteryl esters (Chajek-Shaul et al. 1983). This effect is not related to cholesteryl ester hydrolysis, since nondegradable analogues are transferred to the same extent. These recent data have introduced the novel concept of LPL as a lipoprotein receptor which may be essential for the translocation of cholesterol into cells.

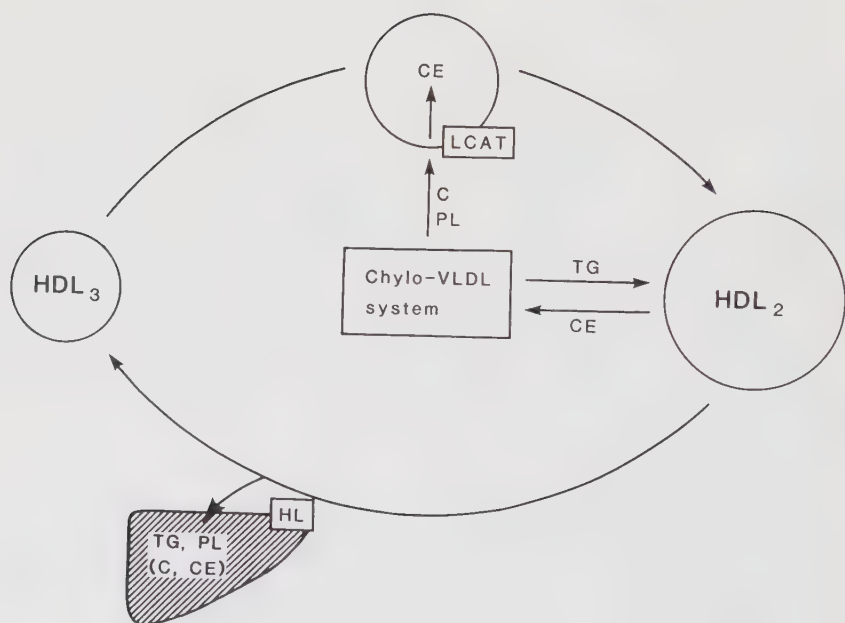


Fig 4. The proposed HDL cycle in man. By the action of LCAT, successive accumulation of cholesteryl esters occurs in HDL₃, which are thus transformed to HDL₂. HDL₂ is also enriched in triglycerides due to an exchange for cholesteryl esters with the chylomicron/VLDL-system. HL is operative in the regeneration of HDL₃, catalyzing the removal of triglyceride and phospholipid from HDL₂; this is accompanied by a passive transfer of cholesterol and cholesteryl ester.

If there is general agreement on the function of LPL, this is not the case for HL. Two major views emerge: a) HL is involved in the degradation of VLDL, IDL and/or chylomicron remnants, and b) HL is involved in metabolism of HDL₂. Evidence for a) may be found in the accumulation of VLDL and IDL after injections of anti-HL antiserum into the circulation (Goldberg et al. 1982). Also, the lipoprotein disturbances noted in the hereditary human deficiency of HL (Breckenridge et al. 1982), may support this view. These interpretations have been challenged by Reardon et al. (1982) who, from kinetic data, conclude that HL is not important in the catabolism of VLDL or IDL. Furthermore, Twu et al. (1984) suggest that the previously used anti-HL preparations may have lacked specificity. However, much evidence is in favour of b). The HL-deficient patients also had elevations of HDL₂. Accumulation of HDL occurs rapidly after the injection of anti-HL antiserum into rats (Kuusi et al. 1979c, Jensen et al. 1980c). Moreover, Kuusi et al. (1980) have demonstrated a strong negative correlation between HL activities and plasma HDL₂ concentrations. It has been suggested that HDL₂ be converted to HDL₃ by the action of HL, and that HL would be a prerequisite for an "HDL cycle" of cholesterol transport. (Nikkilä et al. 1982, see Fig 4). Though this attractive hypothesis is not definitely proved, it could also explain the accumulation of VLDL and IDL when HL activities are low: an inhibition in the flux of surface components may in this situation lead to an inhibition of the lipolytic process with successive accumulation of IDL particles. Studies on LPL-deficient patients have not clarified this issue (Nicol & Lewis 1980, Rao et al. 1982), but data from hypothyroid patients (Valdemarsson et al. 1982) are consistent with view b). As pointed out above, the degradation of triglyceride-rich lipoproteins *in vitro* does not require the presence of HL.

Although the association of HL with HDL transformation seems probable, we do not know exactly how the changes are brought about. The main difference between HDL₂ and HDL₃ is the relatively high content of triglyceride in the former fraction (Valdemarsson 1983); triglyceride is probably acquired via exchange reactions with the chylomicron/VLDL system (Fig 4, cf Lewis 1980). A major function of HL could thus be to dispose of this triglyceride excess, as well as to remove superfluous phospholipid. A further consequence of HL action is the transfer of cholesterol/cholesteryl esters between HDL and tissues (Jansen & Hülsmann 1980a, Lund-Katz et

al. 1982). This translocation appears to be quite extensive, and provides the liver, adrenals, and ovaries with cholesterol for the production of bile acids and steroid hormones. As discussed above in the case of LPL, HL may thus also have a "receptor-like" function.

Relevant characteristics of rat lipoprotein metabolism

The composition and turnover of plasma lipoproteins are markedly different in man and the rat (Fig 3). LDL is the main cholesterol-carrying lipoprotein in human plasma. In the rat, LDL formation is very limited due to the extensive hepatic uptake of remnant lipoproteins (Havel et al. 1976, Faergeman 1977); instead, HDL carries most of the plasma cholesterol in the rat. Rat HDL particles are unimodally distributed, in contrast to the HDL₂ and HDL₃ subfractions of human plasma (Danielsson et al. 1978, 1978a).

Relevant aspects of the physiology of thyroid hormones

The mechanisms of hormone synthesis and secretion by the thyroid gland are known in fairly great detail (Martin 1976 p 189-208, Nunez 1980). The thyroid secretes T_4 and minor amounts of T_3 in response to stimulation by TSH. Over the last decade, the transformation of thyroid hormones has attracted considerable interest, and evidence has accumulated that the extrathyroidal iodothyronine metabolism plays a significant role in the expression of thyroid activity. T_4 may be deiodinated in peripheral tissues to form one of the two isomers, T_3 or rT_3 . Up to 85% of the circulating T_3 is formed by peripheral deiodination of T_4 (Chopra 1981, chapter 6). This process is under metabolic control. During fasting, there is a preferential formation of rT_3 ; on the other hand, feeding leads to increased production of T_3 . (Palmlblad et al. 1977, Balsam et al. 1981). Several drugs and exogenous substances also affect the conversion rate (Cavalieri & Pitt Rivers 1981, see also below). Based on enzymological differences and different subcellular location of T_3 and rT_3 production in vitro, it has been suggested that the formation of the two isomers from T_4 are catalyzed by different enzymes (Chopra 1981, chapter 8). The physiological regulation of T_3 formation seems purposeful as a means of regulating energy output according to metabolic state (see below).

General effects of thyroid hormones

The biological effects of the iodothyronines have been the subject of extensive investigations. It has been demonstrated that rT_3 is virtually inactive in the functions generally associated with thyroid hormones, whereas T_3 is now accepted as the physiologically active thyroid hormone. T_4 may be regarded as a pro-hormone (Chopra 1981, chapter 9). The biological effects of thyroid hormones are exerted via specific nuclear receptors, mainly for T_3 (Oppenheimer et al. 1982). Hormonal stimulation leads to an increase in RNA synthesis and protein production (Tata & Widnell 1966). More recent studies by Oppenheimer et al. (1982) have demonstrated that this stimulation is not indiscriminate: while the livers of hyperthyroid rats contain increased levels of most mRNA species, some are actually present in decreased or unchanged amounts.

A lack of thyroid hormones is associated with decreased energy expenditure; consequently the food intake is reduced. However, the hypothyroid rat evidently sets its intake at a lower level than dictated solely by the deficient production of heat. A decreased growth rate results (Bray 1964, 1978), which is often used as a criterion of hypothyroidism (cf e.g. Kaciuba-Uscilko et al. 1980). It is to be expected that the feeding is increased in hyperthyroid individuals; the rate of weight gain in rats has been reported not to change (Murase & Uchimura 1980), or to decrease (Wilson et al. 1977) following the administration of excess T_4 .

Thyroid hormones also affect carbohydrate metabolism; glucose intolerance and overt diabetes mellitus sometimes accompany hyperthyroidism in man (Werner & Ingbar 1971, p 576). The myxedema of overt hypothyroidism is due to an accumulation of hyaluronic acid in tissues (ibid. p 725-727). This has been attributed to a decreased activity of lysosomal hyaluronidase (deMartino & Goldberg 1981).

Effects on lipid metabolism

It has been known for many years that thyroid hormones influence the metabolism of lipids. Indeed, elevation of plasma cholesterol has been used as a diagnostic criterion of hypothyroidism (Werner & Ingbar 1971, p 285). Walton et al. (1965) demonstrated that this perturbation arises from a decreased catabolic rate of the LDL fraction. It has since been shown that

the LDL receptor is closely regulated by the thyroid activity (Goldstein & Brown 1982). Hypothyroid patients also exhibit elevations of the VLDL and LDL fractions and the elimination rate of triglycerides from plasma is decreased (Nikkilä & Kekki 1972, Valdemarsson et al. 1982, Valdemarsson 1983). The accumulation of VLDL probably results from low LPL activities of both adipose tissue and muscle (Pykälistö et al. 1976, Lithell et al. 1981), since the triglyceride input into the circulation is unaffected by hypothyroidism (Nikkilä & Kekki 1972).

In the hyperthyroid state, LDL concentrations are low due to an increased elimination via the specific LDL receptor (Goldstein & 1982, Haba et al. 1983). There is an increased triglyceride elimination from the circulation, but as the secretion of VLDL is also augmented, plasma triglyceride concentration is usually close to normal (Nikkilä & Kekki 1972).

Thyroid hormones also influence the metabolism of human HDL. A lack of T_3 is usually associated with a slight increase of HDL-cholesterol, due to an increased HDL₂ fraction (Scottolini et al. 1980, Lisch et al. 1982, Valdemarsson 1983; cf. however Agdeppa et al. 1979). This fraction is decreased in hyperthyroid patients (Lisch et al. 1982, Valdemarsson 1983). The relevant mechanisms behind the altered HDL homeostasis are still not firmly established, but there is a relationship to the activities of HL, which are low in hypothyroidism and increase upon hormone substitution (Valdemarsson et al. 1982).

Experimental dysthyroidism in the rat constitutes a different metabolic situation, compared to the human case. The hypothyroid animals down-regulate their energy intake (see above), apparently to a larger degree than is generally the case in humans. In addition, there is a somewhat paradoxical increase in the LPL activities of both adipose tissue and muscle (Shafir & Biale 1971, Kaciuba-Uscilko et al. 1980, Skottová et al. 1983). As there is a simultaneous decrease in hepatic VLDL secretion (Dolphin & Forsyth 1983), the triglyceride levels of plasma tend to be low, as opposed to the hypertriglyceridemia of hypothyroid man. In accordance with man, the activity of the LDL receptor is low (Sykes et al. 1981) resulting in an elevation of this lipoprotein fraction.

Few studies have focused on the chylomicron/VLDL system of hyperthyroid animals. The response in LPL activity to an excess of thyroid hormones, varies, and may depend on e.g. the age of the experimental animals (Wilson et al. 1977).

HL activities are low in hypothyroid rats (Jubelin et al. 1978, Murase & Uchimura 1980); thyroxine treatment does not alter the enzyme activity of post-heparin plasma (Murase & Uchimura 1980). The hormone-deficient rats thus appear similar to hypothyroid patients in their regulation of HL. One would therefore have expected similarities also with regard to HDL levels, but on the contrary Wilcox et al. (1982) have shown that the concentration of this lipoprotein is low in hormone-deficient, and high in hyperthyroid, animals. This discrepancy mainly indicates that the physiological role of HL in the rat is different from that in man.

Innervation of white adipose tissue

Adipose tissue receives nerve fibers running in the perivascular space close to arteries and arterioles; within the tissue the innervation seems to be uneven, establishing rich contacts with some groups of adipocytes, while other areas are virtually devoid of nerve terminals. Rosell & Belfrage (1979) have proposed that this pattern of neural distribution results in the formation of two adipocyte populations that may well respond differently to various metabolic situations. The nerve fibers seem to be mostly, if not exclusively, adrenergic.

The functions of these nerves are known in some detail: they modulate blood circulation and concomitant changes in blood flow are necessary for the full expression of metabolic events in the tissue (Belfrage et al. 1978, Rosell & Belfrage 1979). Another major function seems to be the regulation of lipolysis, i.e. the hydrolysis of storage triglycerides and subsequent release of fatty acids from the tissue (Rosell 1966). Upon stimulation, norepinephrine is released from nerve terminals and interacts with β_1 -adrenoceptors on the adipocyte (Fain & García-Sáinz 1983, Smith 1983). Adrenergic stimulation leads to an activation of adenylyl cyclase and production of cAMP. This substance acts as co-factor for a protein kinase,

which catalyzes the phosphorylation/activation of hormone-sensitive lipase, thereby initiating lipolysis (see Belfrage et al, in press, Strålfors & Belfrage, in press, for reviews). Thyroid hormones potentiate the lipolytic effect of catecholamines, partly by increasing the number of β_1 -adrenoceptors (Fain 1980). There is also definite evidence that catecholamines inhibit lipolysis via α_2 -adrenoceptors (Fain & García-Sáinz 1983). These receptors are absent from rat adipocytes but occur in the hamster and in man (Fain & García-Sáinz 1983). The above reactions may also be elicited by circulating catecholamines. Comparatively little is known about the significance of this hormone pool compared to the locally released norepinephrine.

Innervation of the liver

"Hepatic nerve is really a quite useless appellation.
Of function it tells nothing, serving only for location.
Location, too, is doubtful; of function we know less.
The state of knowledge of these nerves is in a dreadful mess."

Its literary qualities aside, this little poem by Lauth (1980) draws attention to the difficulties encountered in the study of hepatic innervation. A primary problem is the complex anatomy of the liver nerves. The liver receives its innervation through several structures: a major portion of liver nerves enter the organ in the portal area, but there are also direct hepatic branches of the vagus, and some nerves traverse the hepatic ligaments. Total surgical denervation of the liver is thus a tedious task. There is hardly any spatial separation of the sympathetic and parasympathetic systems within the portal area. Within the liver, however, the adrenergic nerves tend to be localized in the interlobular spaces, while choline-esterase-positive neurones are more dispersed (Skaaring & Bjerring 1976, Metz & Forssmann 1980). There are marked species differences in the extent of adrenergic innervation; human liver is richly supplied with catecholamine-positive neurones, while the rat represents a species where this innervation is poorly developed (Moghimzadeh et al. 1982). The difficulties recorded with traditional surgical techniques of denervation have prompted the development of methods for the chemical disruption of neurones. This may be achieved (in the case of the sympathetic system) by intraportal injections of the neurotoxin, 6-OH-dopamine (Lauth &

Coté 1977). The risk of systemic side-effects by this technique cannot be neglected, however.

Efferent innervation

Notwithstanding the difficulties, many investigators have studied the physiological significance of hepatic innervation. One of the best studied functions of efferent nerves is the regulation of glycogenolysis and glycogenesis. Sympathetic neural impulses may increase the activity of glycogen phosphorylase, thereby accelerating hepatic output of glucose (Lautt 1980, Hartmann et al. 1982). The molecular mechanism of phosphorylase activation may be different from that produced by circulating epinephrine and glucagon (Shimazu & Amakawa 1975). Parasympathetic nervous activity stimulates glycogen synthesis by increasing both glucose uptake and glycogen synthase activity. A defective parasympathetic regulation of glucose utilization by the liver has been implicated in the pathogenesis of type II diabetes mellitus (Lautt 1980, 1980a).

Afferent innervation

Much of the hepatic nervous supply is afferent. The liver contains receptors for circulatory pressure/volume, osmotic/oncotic pressure, sodium ion concentration, and painful stimuli (Sawchenko & Friedman 1979, Lautt 1980). Considerable interest has focused on hepatic glucoreceptors which could be operative in the mechanism of satiety (Sawchenko & Friedman 1979). There may also be receptors for other nutrients, e.g. fatty acids (Lautt 1980). Liver denervation may affect food intake in rats, but the effects seem to depend on the operative technique (see, for example, Louis-Sylvestre et al. 1980, Tordoff et al. 1982).

Effects on lipid metabolism

Although the liver has a central position in lipid metabolism, surprisingly little is known about the influence of hepatic innervation in this respect. One of the few studies available (Shanygina et al. 1981) demonstrates alterations in both cholesterol biosynthesis and plasma lipoprotein spectrum after hepatic denervation in rats. Different responses were noted after sympathectomy (celiac neurotomy) and parasympathectomy (vagotomy).

Aims of the present study

1. To study the influence of thyroid state on the LPL and HL activities and lipoprotein profiles, in patients covering the full spectrum of thyroid function from hypo- to hyperthyroidism.
2. To investigate the associations between altered enzyme activities and lipoprotein spectra.
3. To further elucidate the differential effect of hyperthyroidism on activities of LPL and HL in man.
4. To describe the activities of LPL and HL in adipose and heart tissues of hypo- and hyperthyroid rats, and to delineate mechanisms behind the observed changes.
5. To separate primary (direct) effects of thyroid hormones from secondary ones, brought about by concomitant changes in food intake.
6. To compare the effects of T_3 and T_4 on HL regulation.
7. To study the significance of local innervation in the regulation of the activities of adipose tissue LPL, and of HL.

Methodological considerations

Preparation and assay of LPL and HL

Established techniques were used to prepare LPL from heparin eluates (Nilsson-Ehle 1974a) and acetone-ether powders (Nilsson-Ehle et al. 1972) of adipose tissue. The latter method was also used to prepare LPL activity from adipocytes, isolated by collagenase treatment (Nilsson-Ehle et al. 1976). Serum-stimulated LPL secretion from these cells was assessed by the technique of Stewart & Schotz (1973).

We have developed a simplified technique of measuring HL activity in homogenates of rat liver (paper IV). After homogenization of the tissue in a heparin-free buffer, the homogenate is centrifuged at 4,000xg for 10 minutes and the supernatant, containing cytosol and floating fat, is discarded. The pellet (containing microsomal fractions) is resuspended in buffer containing heparin (15U/ml) and is assayed for HL activity. The two-step procedure increases the yield of enzyme activity (Table II), probably due to the elimination of lipid from the second supernate. Dilution with endogenous triglyceride would decrease the specific radioactivity of the substrate, and produce falsely low enzyme activities. The operational definition of HL activity is based on heparin releasability and enzymatic characteristics as defined by the assay system (see below). Our data conform well with those of Cordle et al. (1983), who used a more laborious technique of stepwise ultracentrifugation.

We have also studied the heparin-induced release of HL from cultured rat hepatocytes (paper V). Hepatocyte culture is a well established technique (see e.g. Florén & Nilsson 1978). Briefly, these experiments include culture of hepatocytes from hypo- and euthyroid rats for 24h, followed by exposure to T_3 or T_4 for another 24h. HL activity was then assessed by heparin-stimulated release and enzyme assay. Fetal calf serum is a standard ingredient of culture media which facilitates cell attachment to the culture surface. However, due to significant concentration of thyroid hormones, we eliminated the serum from the medium during hormone stimulation. There was no evidence of increasing cell detachment from the surface. Pilot experiments demonstrated that comparatively high heparin concentrations (100 U/ml) were necessary for maximal HL release (paper V).

Table II

HL activities measured in rat liver homogenates. Two experiments with different animals are shown. Pieces of tissue were either homogenized directly in the presence of heparin, or processed by the two-step procedure described in paper IV. Data are given as mU/g tissue weight, and compared to those of Cordle et al. (1983).

<u>Experiment</u>	1	2	Cordle et al. 1983
<u>One-step preparation:</u>			
(15U heparin/ml)	-	70	-
<u>Two-step preparation:</u>			
First incubation	85	91	-
(no heparin)			
Second incubation	234	119	275 [±] 132 (mean [±] SD)
(15U heparin/ml)			

Assays for LPL and HL were done as described by Nilsson-Ehle and Ekman (1977). These methods take advantage of the fact that a phosphatidylcholine emulsion of triglyceride, prepared in the absence of albumin, is selectively attacked by LPL; in the HL assay, specificity is ensured by the total inhibition of LPL by 1M NaCl. A particular advantage is the easy separation of the two enzyme activities in post-heparin plasma by the use of these techniques (Ehnholm et al., in press). As in all lipase assays, highly purified triglyceride is a prerequisite for satisfactory accuracy. In addition, the precision of LPL assay deteriorates in the presence of partial glycerides (Nilsson-Ehle, in press; cf Gamlen & Muller 1980). We continuously monitored the purity of both labelled and unlabelled triolein in order to optimize assay performance.

Selection of patients and volunteers

The patients in paper I were divided into three groups, overt hypothyroidism, subclinical hypothyroidism and hyperthyroidism, and were compared to a matched euthyroid control group. Patients with overt hypothyroidism had depressions of serum T_3 levels and increased TSH concentrations. Subclinical hypothyroidism was defined by increased TSH levels with normal T_3 concentrations. Elevated T_3 concentrations were used as a criterion of hyperthyroidism. The longitudinal study (paper II) included men of similar age; this should result in a relatively low interindividual variation. Experimental hyperthyroidism was induced by T_3 rather than T_4 in order to eliminate differential rates of conversion (see above) as a source of variation in the biological response.

Experimental dysthyroidism in the rat

There are several ways of producing experimental hypothyroidism in animals. Surgical thyroidectomy is attractive, since the pre-formed hormones are removed with the gland; therefore, the effect is fairly rapid. However, accidental elimination of, or damage to, the parathyroids may be difficult to avoid. Treatment with ^{131}J is also possible, but the high dose needed (30-50 MBq/animal) causes environmental problems. We therefore chose to administer PTU, which effectively blocks the synthesis of iodothyronines. An additional advantage of this compound is its inhibition of peripheral T_4 conversion to T_3 in most organs (Cavalieri & Pitt-Rivers 1981). PTU may be administered by different routes: we have found it most convenient to add it to the drinking water of the animals. In our first experiments (paper III)

we used a high dose of 1 g/l. This was not accepted by some rats and we therefore decreased the dose to 0.1 g/l, which is also adequate to produce hypothyroidism (Cooper et al. 1983). Besides the decreased food intake, this regimen had no apparent side effects.

ITU, which is one of the most potent inhibitors of hepatic T_3 formation so far described (Chopra et al. 1982), was used to differentiate between the effects of T_3 and T_4 in the in vitro study, reported in paper V. We have also administered ITU to rats in vivo (Table III). In this experiment, the most marked effect of ITU administration was an elevation of serum rT_3 levels, which increased almost tenfold. Serum T_4 concentrations doubled, but T_3 remained essentially normal. The elevation of rT_3 is secondary to enzyme inhibition, since T_4 and rT_3 appear to be deiodinated by the same enzyme (Chopra 1981, chapter 6). Similarly, the block in T_4 degradation could explain the increase of serum T_4 levels. However, since T_3 concentrations were not decreased, one must consider that e.g. thyroid hormone secretion could be altered. There may also be an increase in the thiouracil-insensitive T_4 -deiodination of extrahepatic tissues (cf Cavalieri & Pitt-Rivers 1981). From a physiological point of view, the ITU-treated animals seem to be essentially euthyroid. Their food intake is not depressed and may even be increased. Also, their plasma lipoprotein pattern is not different from that of euthyroid animals.

To avoid possible interference by different rates of T_4 deiodination (see above), T_3 was used to produce experimental hyperthyroidism in the rat. Pair-feeding experiments are difficult to design, since hyperthyroid rats eat more than controls. We have solved this problem by using two groups of T_3 -treated rats given either 20 g, or a free amount, of food daily. The euthyroid controls also received 20 g per day, an intake set just below spontaneous food consumption to ensure that all is eaten (paper IV).

Table III

Effects of ITU and PTU on thyroid hormones, enzyme activities, plasma lipoproteins, and food intake in rats. Both drugs were administered in the drinking water (0.1 g/l) for two weeks. Data are given as mean $\pm$ SD. rT_3 concentrations were measured by a radioimmunoassay kit from Serono-Biodata, Coinsins, Switzerland.

Group	ITU	PTU ¹	Euthyroid ¹
T_3 (nM)	1.23 $\pm$ 0.13	< 0.5	1.07 $\pm$ 0.12
rT_3 (nM)	2.09 $\pm$ 0.80 ^a	0.16 $\pm$ 0.04	0.22 $\pm$ 0.04
T_4 (nM)	110.8 $\pm$ 8.7 ^a	< 20	51.4 $\pm$ 6.5
Tissue HL activity (mU/mg protein)	14.5 $\pm$ 2.0	7.2 $\pm$ 1.1	13.4 $\pm$ 1.6
Adipose tissue LPL (mU/mg protein)	0.60 $\pm$ 0.11 ^a	0.94 $\pm$ 0.70	0.42 $\pm$ 0.17
Heart LPL ("")	1.56 $\pm$ 0.72	1.12 $\pm$ 0.31	1.20 $\pm$ 0.66
Serum cholesterol (mM)	1.92 $\pm$ 0.32	2.53 $\pm$ 0.22	1.72 $\pm$ 0.22
HDL-cholesterol (mM)	1.24 $\pm$ 0.12	1.07 $\pm$ 0.09	1.04 $\pm$ 0.13
Food intake (g/day)	28.0 $\pm$ 1.9	19.0 $\pm$ 1.4	23.3 $\pm$ 3.8

¹ = data from paper IV

a = significantly different compared to the euthyroid group

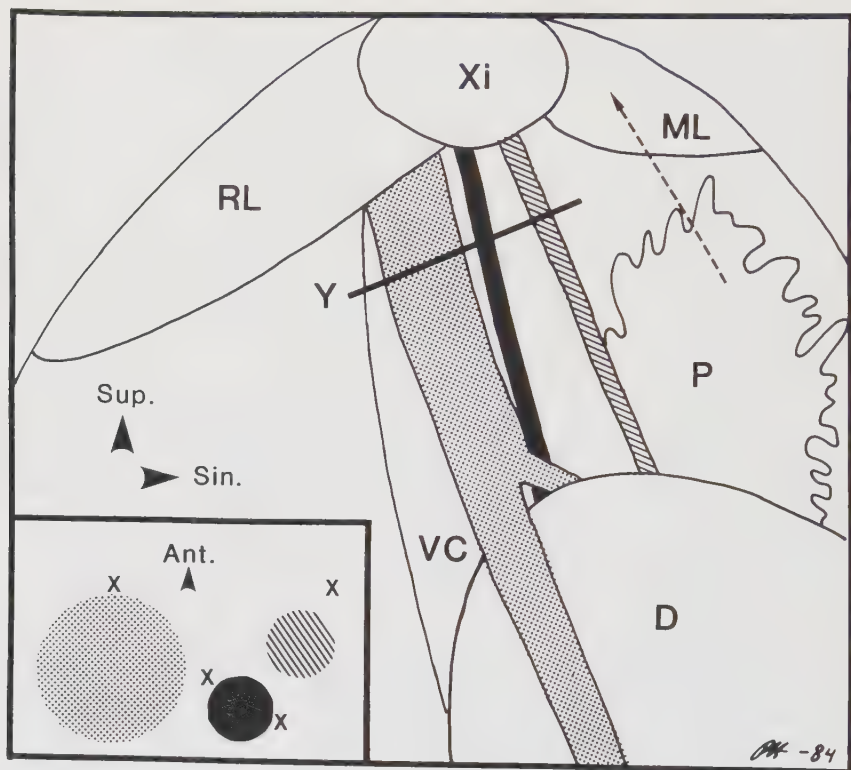


Fig 5. Frontal view of the operating field during portal denervation of the rat liver. The duodenum (D), pancreas (P), and intestines have been reflected to the left (sin.) The positions of the portal vein (shaded), hepatic artery (black), and bile duct (hatched) are also indicated in a transversal plane, Y (insert), where the approximate positions of major nerve trunks are indicated by crosses. The dashed arrow shows the course of vagal branches entering the liver directly (not visible). Ant.= anterior. Sup.= superior. ML = median liver lobe. RL = right liver lobe. VC = inferior vena cava (deeper location) Xi = xiphoid process.

Techniques of denervation

Unilateral denervation of the epididymal fat in male rats has the advantage of providing a paired experiment, where the contralateral fat pad is the control (paper VI). Modern microsurgical techniques offer the possibility of a distal (and specific) organ denervation even in small animals. The visualization of nerves is greatly improved by the application of a few drops of 1% toluidine blue, after cutting the peritoneum. The dye also renders the lymphatic vessel visible, preventing accidental injuries.

Fig 5 shows the relevant anatomical structures of hepatic denervation (paper VII). The microsurgical technique is described elsewhere (Holmin et al. in press). Again, toluidine blue is useful in visualizing the nerves. It should be noted that several direct vagal branches to the liver are easily distinguished, and spared. It is possible to remove the major part of the adrenergic nerve supply; some vagus neurones are inevitably included (see above). After the operations, neurone degeneration was allowed to proceed for one week, which normally ensures total disappearance of nerve terminals (Lautt 1980). Measurement of tissue norepinephrine concentration demonstrated a virtually total denervation of the adipose tissue, while about 15% of the adrenergic supply remained in the denervated liver.

Plasma lipids and lipoproteins

Established methods have been used to measure plasma concentrations of triglyceride and cholesterol. Determinations of HDL-cholesterol, -phosphatidyl choline and -triglyceride were done after non-HDL-lipoproteins had been precipitated by dextran sulfate and MgCl_2 (Danielsson et al. 1978). LDL-cholesterol was calculated by the formula of Friedewald et al. (1972), modified to SI units. It should be noted that this variable includes the cholesterol of the IDL fraction, which normally is very small but increases in hypothyroid patients (Valdemarsson 1983).

Results & Discussion I: The effects of altered thyroid state in man and the rat

Regulation of LPL in man

Hypothyroid patients have a decreased LPL activity in postheparin plasma (paper I). This is in agreement with the decreased activities found in adipose tissue and muscle (Pykälistö et al. 1976, Lithell et al. 1981). The changes are seen only in the patients classified as overtly hypothyroid, i.e. with depressed serum levels of T_3 . Subclinical hypothyroidism (i.e. elevated TSH levels in serum, but T_3 concentrations within reference limits) is associated with an essentially normal enzyme activity. Similar results have been found by Lithell et al. (1981).

The LPL activities of hyperthyroid patients were not different from those of euthyroid controls. However, evaluation of cross-sectional studies is influenced by the biological variation in the sample which may obscure minor differences between groups. In longitudinal studies, however, the inter-individual variation tends to dampen out, since each subject represents his own control. We therefore undertook such a study of the effects of T_3 administration (paper II); the selection of volunteers should provide a homogeneous group. Even with this experimental design, we did not detect any significant change in LPL activities of post-heparin plasma. Further studies are needed to clarify whether this is a true lack of response; it is possible that opposite changes in the enzyme activities of e.g. muscle and adipose tissue could cancel out one another.

LPL regulation in the rat

In paper III we have investigated possible mechanisms behind the increased LPL activity in adipose tissue of hypothyroid rats. In this context, it is important to choose a proper group of control animals. Due to the reduced weight gain associated with hypothyroidism (see above), we have found it necessary to include both age-matched and weight-matched control rats. LPL activity of isolated adipocytes was only marginally increased by hypothyroidism. In contrast, the enzyme activity of the whole tissue was markedly higher, especially as measured in heparin eluates. This method is thought to reflect mainly the amount of extracellular enzyme. LPL secretion was also increased, but more moderately than the heparin-elutable

activities. These data indicate that rat hypothyroidism causes an accumulation of extracellular LPL. Our results also raise the possibility that the half-life of extracellular LPL may be a point of regulation of the enzyme activity (cf Cryer 1981). The mechanisms responsible cannot be specified. It is possible that an extracellular LPL-degrading system (see above) is inhibited by the lack of thyroid hormones. The amount of LPL-binding glycosaminoglycans may also change (cf de Martino & Goldberg 1981).

The hypothyroid rats of paper III had increased enzyme activities in heart tissue. However, we could not reproduce this effect in paper IV, possibly due to differences in the experimental designs, such as the dosage of PTU (see above). Previous studies using different techniques of enzyme preparation have also given inconclusive results (Shafrir & Biale 1971, Kaciuba-Uscilko et al. 1980).

Comparison of data from the different control groups makes it possible to separate the effects of alterations in food intake from those of hypothyroidism per se. Evidently, the decreased long-term food intake contributes to the elevations of LPL activities induced by hypothyroidism. The differences noted between animals studied in the fasted and fed states, indicate that the effects of the reduced long-term food intake are operative mainly during post-prandial periods. Besides the information on hypothyroidism, this study yielded interesting information on the effects of prolonged semi-starvation. Evidently this treatment augments the normal response of adipose tissue LPL activity to feeding. Similar "overshoots" have recently been reported after a three-day fast (Fried et al. 1983). Apparently, the increase in enzyme activity is mainly due to an accumulation of extracellular LPL.

The spontaneous changes in feeding are also of great importance for enzyme regulation in hyperthyroid rats (paper IV). In T_3 -treated animals, pair-fed to euthyroid controls, LPL activities (expressed per mg of protein) decrease in both adipose and heart tissues. If spontaneous hyperphagia was not prevented, the enzyme activity of adipose tissue normalized, while it was still low in heart. When comparing these data to the earlier literature, it is to be noted that other authors have expressed the enzyme activities relative to wet weight. This may be significant, since myocardial hypertrophy

accompanies hyperthyroidism (cf Alousi & Mallov 1964, Wilson et al. 1977, Kaciuba-Uscilko et al. 1980).

Regulation of HL in man

The importance of hypothyroidism for HL regulation has previously been shown by our group (Valdemarsson et al. 1982). Paper I extends this observation, and demonstrates an effect of thyroid hormones on HL activity over the whole spectrum of thyroid function. There is a strong correlation between T_3 concentration and HL activity, which suggests a direct hormonal effect on the enzyme. HL activities of patients with subclinical hypothyroidism were not different from those of euthyroid controls. This is consistent with the essentially normal T_3 levels of these patients. Paper II provides further support for a direct effect of T_3 . An increase of HL activity of post-heparin plasma was seen upon hormone administration to healthy men already after 24 hours. The increase in enzyme activity roughly paralleled the increment in serum T_3 levels.

Regulation of HL in the rat

HL activity of liver tissue displayed a low biological variation (paper IV). However, the activity levels were different in the two rat batches. We cannot explain this by differences in feeding, or the handling or killing of the animals. One may therefore assume that different litters may have different HL activities, possibly genetically determined (cf Agbedana et al. 1982). In contrast to LPL, HL regulation by thyroid hormones seems similar in man and the rat (paper IV). The enzyme activity in hypothyroid rats was decreased by about 50% (cf Murase & Uchimura 1980), which apparently was not related to the decreased food intake. Increased HL activities were seen in all T_3 -treated animals. However, the spontaneous hyperphagia of hyperthyroidism accentuated the direct stimulatory effects of the hormone excess.

Paper V demonstrates that thyroid hormones directly affect HL release from cultured rat hepatocytes. The inhibition of the effect of T_4 (but not of T_3) by ITU suggests that the effect is exerted by T_3 . Cells from hypothyroid rats responded more vigorously than those from euthyroid animals. We are presently conducting studies to further characterize the cellular mechanisms

by which T_3 exerts its effect. Similar studies on human material must await better techniques for cell culture.

Lipoprotein alterations in man

In clinically hypothyroid patients (paper I), we noted moderate increases in plasma levels of triglycerides (reflecting the amount of VLDL) and marked elevations in LDL-cholesterol (similarly reflecting IDL and LDL). The changes in VLDL and LDL are well documented and are mostly due to an inhibition of the removal of these lipoproteins, rather than an increased synthesis (Walton et al. 1965, Nikkilä & Kekki 1972, Abrams & Grundy 1981, 1981a, Thompson et al. 1981). We have recently shown that the IDL pool increases in hypothyroidism and rapidly disappears upon hormone substitution (Valdemarsson 1983). In this cross-sectional study we did not find any significant change in HDL-cholesterol in the hypothyroid patients; however, a significant reduction of HDL-cholesterol upon substitution therapy (Valdemarsson et al. 1982) indicates that there is indeed a tendency towards increased HDL concentrations in hypothyroidism. We have demonstrated that the major changes occur in the HDL₂ subfraction (Hansson et al., submitted). In this situation, the low LPL and HL activities seem to balance each other, thus maintaining essentially normal HDL concentrations. The patients with subclinical hypothyroidism had a moderate but significant elevation of LDL-cholesterol. Since the other lipoprotein variables were essentially normal, the increase is probably due to a small inhibition of LDL clearance, possible together with a limited expansion of the IDL pool.

Hyperthyroidism, both clinical and experimental (papers I,II) lead to essentially the opposite changes, including decreases of both LDL-cholesterol and HDL-cholesterol. The low LDL levels are probably due to increased catabolism (Walton et al. 1965, Chait et al. 1979, Haba et al. 1983). Both production and removal of VLDL-triglyceride is increased in human hyperthyroidism (Nikkilä & Kekki 1972), and these changes seem to cancel out one another. The low HDL concentrations can readily be attributed to the high HL activities.

These data may be interpreted in the general model of lipoprotein metabolism: In hypothyroidism, low LPL activities lead to an impaired degradation of triglyceride-rich particles. This causes an elevation of VLDL

levels, and concomitantly a reduced transfer of surface components to HDL. The decreased input into the HDL system is balanced by a decreased elimination, due to the low activities of HL and LCAT. The appearance of IDL may thus be the result of a back-up in the flux of surface components through the HDL system to the liver. Finally, the low activity of the LDL receptor leads to an increased plasma pool of LDL.

In hyperthyroidism, the slightly higher plasma triglyceride levels seem due to an overproduction of VLDL (Nikkilä & Kekki 1972). The transfer of surface components to HDL may be slightly increased, but this is outweighed by the marked increases in HL and LCAT activities (cf Valdemarsson 1983), leading to a significant decrease in HDL. Increased LDL catabolism via the specific receptor explains the lowered LDL concentrations.

Characteristics of the lipoprotein metabolism of dysthyroid rats

The deficit or excess of thyroid hormones in the rat produces changes of lipoprotein metabolism that only partly resemble the changes in man. Hypothyroid animals have increased plasma levels of cholesterol (paper III, IV), which are accounted for by an increase of non-HDL-cholesterol. This is in accordance with data in man. However, triglyceride levels are decreased, contrary to clinical observations (see above). This is probably due to a combined effect of decreased VLDL production (Dolphin & Forsyth 1983) and increased LPL activities of muscle and adipose tissue (see above). There was a small increase in HDL-cholesterol of rats receiving the higher PTU dose (paper III); this finding was not reproduced using the lower dose, although these rats were also hypothyroid (paper IV). We thus cannot entirely exclude an additional effect of PTU on HDL metabolism. Wilcox et al. (1982) have noted a decrease in HDL-cholesterol following PTU treatment to rats. The variability in HDL response indicates that other factors, e.g. dietary regimen, are more important than the hypothyroid state as such.

Non-HDL-cholesterol was uniformly depressed in both groups of T_3 -treated rats. This is probably due to an increased catabolism of LDL (cf Sykes et al. 1981), similar to the situation in man. In contrast to our clinical findings there was no tendency to a decrease in HDL-cholesterol. On the contrary, the free-eating hyperthyroid animals had clearly increased HDL concentrations. This is consistent with the report of Wilcox et al. (1982),

who also described an increased hepatic secretion of apo AI. Again, these data indicate that changes in feeding are of greater importance for HDL concentrations than thyroid state per se.

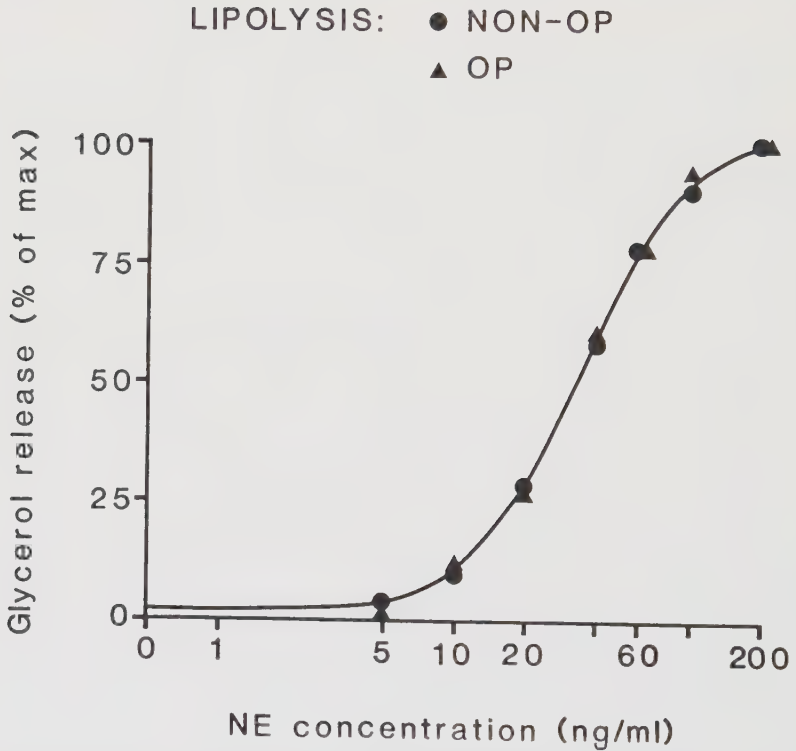


Fig 6. Dose-response curve of the lipolytic response to norepinephrine by adipocytes from a denervated (triangles) and from the control (circles) fat pad. The animal was sacrificed one week after the operation. Adipocytes were isolated by collagenase treatment (see text), suspended in Krebs-Ringer-HEPES buffer, and exposed to various norepinephrine concentrations for 1h at 37°C. The adipocytes were then separated from the medium by flotation through dinonyl phthalate, and glycerol content of the cell-free buffer was assayed by a kit from Wako Ltd, Osaka, Japan. No endogenous norepinephrine was detectable in the denervated tissue.

Results & Discussion II: Lipase activities after denervation

Adipose tissue

The microsurgical procedure used in paper VI produces virtually complete adrenergic denervation with a success rate of 80%. With proper precaution, there are no signs of vasospasm or other circulatory reactions to the operation. LPL activities in denervated adipose tissue was not different from that of the contralateral fat pad. The heparin-elution technique for LPL preparation seems to be quite sensitive in detecting responses to different stimuli (cf paper III). Judging from these data, it is unlikely that LPL activity of white adipose tissue is directly controlled via the adrenergic innervation.

We have also investigated the effects of denervation on lipolysis. Somewhat surprisingly, denervation does not seem to alter the lipolytic response of isolated adipocytes (Fig 6). These results seem to contrast to earlier studies in dogs (Rosell 1966). However, the apparent controversy could be related to the presence of both innervated and non-innervated adipocytes (Rosell & Belfrage 1979). If the latter pool is larger, present methodologies may be unable to detect an altered lipolytic response of the truly denervated adipocytes only. Alternatively, the influence of locally released nor-epinephrine may be masked by effects of circulating catecholamines. The elimination of circulating catecholamines by concomitant adrenalectomy may allow the testing of this hypothesis.

Liver

The microsurgical technique used in paper VII provides a good adrenergic denervation in about 90% of the operated animals. The advantage of microsurgery lies in the possibility to perform a more selective and reproducible denervation than has previously been possible. The selectivity in terms of adrenergic/cholinergic denervation can never be complete; however the present technique is better also in this respect, since the direct vagus branches are kept intact. No peptidergic nerves (immunoreactive to gastrin, cholecystokinin, vasoactive intestinal peptide, substance P, somatostatin or enkephalin) were resected (Holmin et al., in press).

HL activities in denervated liver tissue was about 20% higher than in the control groups. Our experimental design did not give us any clue

as to the mechanisms behind the increased enzyme activities; food intake and other variables, including biochemical liver tests, were not affected by denervation. The interaction between hormonal and neural regulation of HL is currently under investigation.

General discussion

A major part of this thesis deals with the impact of thyroid hormones on lipid metabolism. From the clinical point of view, thyroid disease is common enough to constitute a significant health problem, and manifestations of dyslipoproteinemia, e.g. cardiovascular disease, are of clinical importance in hypothyroidism. From the biochemical perspective, thyroid dysfunction provides unusually pure model situations for perturbations of lipid metabolism in man.

The present studies originate in clinical descriptive investigations (paper I, Valdemarsson 1983) which are followed up by experimental studies in man (paper II) and in the rat (papers III, IV, V). These investigations were designed to elucidate the mechanisms behind enzyme alterations in thyroid dysfunction, and to register the effects of altered enzyme activities on the plasma lipoprotein profile. The selective elevation of HL activities in hyperthyroid patients was considered especially interesting since it represents a situation where the influence of HL on plasma lipoprotein concentrations can be studied without concomitant changes in LPL activity. The rapid fall in plasma HDL concentrations accompanying the rise in the HL activity upon T_3 administration, supports present views on HL as an HDL-degrading enzyme. Taken together with a model situation for a selective increase in LPL activities, such as chronic alcohol consumption (Nilsson-Ehle 1979), which is associated with increased HDL concentrations, these data indicate that HDL levels in man are to a large extent determined by the balance between HL and LPL activities.

Since a more detailed delineation of biochemical mechanisms cannot be performed in man with existing methodologies, further studies were performed in the rat. Extrapolation to the human situation from such data may, however, be done only in the light of thorough knowledge about similarities and differences in the metabolism of the two species. From these investigations,

it seems clear that marked differences between man and rat occur both with regard to thyroid regulation of LPL and HL activities and with regard to the impact of changes in enzyme activities on the plasma lipoprotein profile.

While it is generally assumed that the function of LPL is similar in man and in the rat, marked differences are found in the regulation of enzyme activities. Paper III shows that hypothyroidism in the rat, in contrast to man, leads to increased enzyme activities in adipose tissue. The use of different enzyme preparations allowed a tentative location of the sites for the altered LPL regulation; probably, the development of immunohistochemical methods (cf Olivecrona & Bengtsson 1983) will provide even more detailed information in this area.

Judging from papers III and IV, the rat seems to be a better animal model for the study of LPL function rather than of LPL regulation. The opposite seems true for HL: the response of enzyme activity to dysthyroidism is similar (papers I, II, IV, V) whereas the consequences of an altered HL activity on plasma HDL concentrations are different (I, II, IV). Several explanations for these phenomena are possible. The differential impact of HL activities on plasma lipoprotein metabolism does not necessarily imply that the enzyme has different functions in the two species. Rather, it seems that other factors influencing HDL concentrations override the direct impact of HL activity. For example, Wilcox et al. (1982) have demonstrated an increased secretion of apo AI (and thus presumably of HDL) from perfused livers of hyperthyroid rats. Triiodothyronine thus seems to affect both the production and degradation of this lipoprotein class in the rat. We do not know if a similar overproduction occurs in hyperthyroid patients, but it seems that HDL levels in dysthyroid man can be explained mainly by alterations in HL and LPL activities (Valdemarsson 1983).

Papers III & IV also draw attention to another mechanism of enzyme regulation by thyroid hormones, i.e. via their influence on food intake. This

aspect has so far received comparatively little attention. The hormonal effects on enzyme activities are modulated to a different extent by feeding, which may be outlined as follows:

	Hypo/hyperthyroidism		
	Heart LPL	A.t. LPL	HL
Direct hormonal regulation	+?/+	+/(?)	+/+
Modulation via feeding	-/-	+/+	-/+

A.t., adipose tissue

The pronounced effects of feeding on enzyme regulation in adipose tissue are probably related to its function as an energy depot. By contrast, it seems logical that the nutritional state is less important in the heart which has rather constant energy demands. The influence of different food composition is not addressed here. The literature in this field is very limited, but it is likely that changes in food composition may also modulate the metabolic effects of hormonal stimulations (Weisenberg-deLorme & Harris 1975, Hülsmann et al. 1977, Agbedana et al. 1979, Cryer 1981).

Medical textbooks generally refer to altered eating habits of dysthyroid patients en passant, and this aspect seems to have received comparatively little attention clinically. Little is known about possible food preferences in clinical hypo- and hyperthyroidism. Also in a broader perspective, few clinical studies on LPL or HL regulation have taken measures to define possible primary and secondary (nutritional) effects. The marked effects of food intake on hormonal action in the rat indicates that this would be an important area for future studies in man.

Several further lines of investigation emerge from the present studies. One logical development would be to repeat the longitudinal experiment of paper II and quantitate the acute changes in the lipoprotein spectra after T_3 administration, preferably by zonal ultracentrifugation. A decrease of HDL_2

would further strengthen the association of HL with the metabolism of this lipoprotein class. An adequate control of food intake would probably be essential even in such a short-term experiment. The rat hepatocyte culture (paper V) seems to be a suitable model for a "chemical dissection" of T_3 effects on HL processing. Model substances as cycloheximide (protein synthesis inhibitor), colchicin (microtubule depletor) and tunicamycin (glycosylation inhibitor) may easily be added to the system.

Investigations of the role of local innervation on LPL/HL regulation have been hampered by the lack of good surgical procedures. Our own primary effects to denervate, by truncal sympathectomy, the adipose tissue were not successful; the application of microsurgical techniques (cf Silber 1979) resulted in a better denervation achieved at a more distal location (paper VI). The use of less specific techniques such as isogenic tissue transplantation (Ikeda et al. 1983) or vagotomy (Courtney et al. 1983) carries the risk of unwanted side effects or confounding systemic effects. The possibility of paired experiments in the case of adipose tissue is a further advantage. This experimental model seems to have a great potential for studies on neural regulation of adipose tissue metabolism and physiology, and for investigations on the interaction between neural and hormonal influences.

The results of liver denervation probably depend much on the exact operative technique. Again, the use of the operating microscope greatly aids the localization of the nerves, and increases the specificity and reproducibility of the surgical procedure. The response of rat HL activity to liver denervation raises the question if nervous HL regulation is also present in man. During partial hepatic resection (cf Blumgart 1983) hilar denervation is sometimes inevitable (T. Holmin, personal communication) and little is known about its possible long-term consequences on liver metabolism. The use of adrenergic agonists/antagonists is accompanied by alterations in the lipoprotein profile (see e.g. Hooper et al. 1981); the exact mechanisms are little studied, but could well include altered synthesis or transformation of lipoproteins by the liver.

Further experiments in the could confirm and extend the preliminary observations of paper VII. It should be possible to repeat the previous study of

Shanygina et al. (1981) with a more precise technique. The amount and composition of rat plasma lipoproteins after hepatic denervation will have to be investigated. Also in more general terms, the adrenergic denervation may serve as a tool for investigation of neural influence on hepatic metabolism.

Acknowledgements

First of all, my thanks go to Peter Nilsson-Ehle, my mentor. He introduced me into the field of lipoproteins and lipases, and has provided continuous guidance and inspiration throughout the course of this work. I have been especially lucky to share his knowledge on how to do proper research and how to report results properly. Professor Per-Arne Öckerman and the Board of the Department kindly placed facilities at my disposal.

I am especially grateful to the people of our research group. Lilly Berlin and Gerd Nilsson have taught how to behave about a lab: most of the studies would not have been possible, had it not been for their dedicated assistance. Ivar Larsson always seemed to bring me my photographs before I had delivered the proofs. I am especially grateful for his lessons on ultracentrifugation. The discussions with Sten-Erik Bäck and Gunnar Nordin have been extremely valuable.

It has been most pleasant and fruitful to collaborate with drs Stig Valdemarsson and Pavo Hedner. I am now aware that a clinical chemist cannot work in an ivory tower: close contact with the clinical world is essential.

The study of paper V depended totally on the co-operation by drs Elmo Jensen and Claes-Henrik Florén. I am very grateful to them for introducing me into the mysteries of cell culture.

After a few fumbling hours at the microscope, I realized that you need microsurgeons to do microsurgery. Fortunately, such people were close at hand. Without the dedication and enthusiasm of Torsten Holmin, Carl-Magnus Kullendorff, and Jan Lindfeldt, papers VI and VII would still have been only ideas. I am also grateful to dr Mats Ekelund and to

Professor Anders Björklund for sharing their knowledge of neurobiology with me.

Ingrid Stedman finally won the battle with the word processor so that the text appeared the way WE wanted it to. Thanks for all the overtime work! I am grateful to Academic Press Inc., Acta Endocrinologica, Elsevier Scientific Publishers, and Georg Thieme Verlag for permissions to reproduce the printed papers.

Thanks are due to Per Belfrage (Lund), Paavo Kinnunen (Helsinki), and Louis Smith (Houston) for providing advance copies of their reviews, and to Jan Rollof for providing the "Joker" (see back cover). Finally, I thank my father, family, and friends for inspiring and reassuring me at all times.

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Relations between thyroid function, hepatic and lipoprotein lipase activities, and plasma lipoprotein concentrations

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Abstract. Lipoprotein concentrations and activities of lipoprotein lipase (LPL) and hepatic lipase (HL) were measured in 70 subjects with thyroid function ranging from overt hypothyroidism over subclinical hypothyroidism and euthyroidism to hyperthyroidism.

In parallel with serum T₃ (S-T₃) concentrations increasing from low in hypothyroidism to high in hyperthyroidism there were gradually higher HL activities over the full spectrum of thyroid function, accompanied by decreasing levels of total and low density lipoprotein (LDL) cholesterol. High density lipoprotein (HDL) cholesterol was lower ($P < 0.05$) in hyperthyroidism than in euthyroidism but not significantly changed in the hypothyroid groups. HL was correlated to S-T₃ ($r = 0.77$, $P < 0.001$), LDL cholesterol to log S-T₃ ($r = -0.76$, $P < 0.001$), and LDL cholesterol to log HL ($r = -0.55$, $P < 0.001$).

The activity of LPL was decreased ($P < 0.001$) in overt hypothyroidism compared to euthyroidism but, in contrast to HL, the activity of LPL was not increased in hyperthyroidism. The plasma triglyceride (P-TG) concentration was elevated ($P < 0.01$) in overt hypothyroidism but not significantly changed in subclinical hypothyroidism or in hyperthyroidism. The LPL activity was correlated to log S-T₃ ($r = 0.45$, $P < 0.001$), P-TG to log S-T₃ ($r = -0.37$, $P < 0.01$) and P-TG to log LPL activity ($r = -0.71$, $P < 0.001$).

Our results demonstrate that thyroid hormones influence HL and LPL activities in different ways, suggesting different mechanisms of action. Changes in HL activity seem to be an important mechanism for the disturbance of cholesterol metabolism in thyroid dysfunction while the thyroid hormone influence on LPL seems to be of importance mainly for the disturbance in triglyceride metabolism.

When patients with hypothyroidism are treated with L-thyroxine an increase in lipoprotein lipase (LPL) and hepatic lipase (HL) activities has been found to parallel the normalization of their elevated total and LDL cholesterol levels (Pykälistö et al. 1976; Lithell et al. 1981; Abrams et al. 1981; Valdemarsson et al. 1982). In hypothyroid patients under treatment we also found the increase in S-T₃ to be significantly correlated to the increase in HL activity and to the decrease in LDL cholesterol as well (Valdemarsson et al. 1982). These results indicate that LPL and HL are of pathogenic importance for the dyslipoproteinaemia in hypothyroidism. However, the nature of a possible general association between thyroid hormone concentrations, lipase activities, and lipoprotein levels can not be determined from effects of treatment of hypothyroidism only, and data on lipase activities in hyperthyroidism are inconclusive (Nikkilä & Kekki 1972; Tulloch et al. 1973; Abrams et al. 1981). Therefore, we investigated these associations in a cross-sectional study of 70 subjects with various thyroid function states ranging from overt hypothyroidism over subclinical hypothyroidism and euthyroidism to hyperthyroidism.

Material and Methods

Patients

Fifty-three (consecutive) patients and 17 ostensibly healthy subjects (doctors, technicians, students) were

investigated after giving informed consent. The investigation was approved by the Ethical Committee of the University of Lund, Sweden. The subjects were allocated into four groups according to their thyroid function: overt hypothyroidism (group 1) with clinical signs of hypothyroidism, elevated serum levels of TSH (S-TSH) and S-T₃ below our lower reference limit; subclinical hypothyroidism (group 2) with elevated S-TSH and S-T₃ within our reference range; healthy subjects (group 3); and hyperthyroidism (group 4) with elevated S-T₃. Details on age, weight, and sex distribution appear in Table 1. It is unlikely that the minor differences between the groups in mean body weight, age and sex distribution would significantly influence our conclusions from data on lipase activities (Huttunen et al. 1976) or lipoprotein concentrations (Barclay 1972). The substitution effects in 14 patients with overt hypothyroidism have been reported elsewhere (Valdemarsson et al. 1982). Their pre-treatment values were included in group 1.

All patients with increased S-TSH concentrations had elevated titres of circulating thyroid antibodies.

None of the subjects was on any drugs or diets known to influence serum lipids or thyroid function. Clinical and laboratory signs of diabetes or liver or kidney disease were absent.

Analytical methods

Methods for determination of thyroid hormone levels, activities of HL and LPL and lipoprotein concentrations have been described elsewhere (Valdemarsson et al. 1982).

Statistical methods

Coefficients of correlation and regression lines for different variables were calculated by the least square method by a computerized programme. Data were also analyzed after transformation in the following ways: $\log x$ vs y , $\log y$ vs x , $1/x$ vs y , $1/y$ vs x , $\sqrt{x}$ vs y , $\sqrt{y}$ vs x and $1/x$ vs $1/y$. Multiple regression analyses were performed by standard techniques (Draper & Smith 1966).

Results

The mean values for the thyroid function variables in the patients with subclinical hypothyroidism (group 2) were intermediate between those of the patients with overt hypothyroidism (group 1) and those of the euthyroid subjects (group 3) and significantly different from both (Table 1). The thyroid function values in the hyperthyroid patients (group 4) were well separated from those of the healthy subjects (Table 1).

Table 1 also summarizes the mean values of lipoprotein concentrations, the activities of HL and LPL and the elimination rate of exogenous triglyceride. There was a continuous decrease in P-cholesterol concentrations from group 1 to group 4, the levels in all groups being significantly different from each other. A similar pattern was found for LDL cholesterol, while HDL cholesterol differed only in the hyperthyroid patients, who had 17% lower group mean concentration than the euthyroid subjects. The P-triglyceride level and IVFTT were significantly changed only in overt hypothyroidism.

The group mean values of HL activities (Table 1) gradually increased from markedly reduced levels in severe hypothyroidism to clearly elevated levels in hyperthyroid patients. In contrast, LPL activity was clearly lowered only in severe hypothyroidism, while the activities in subclinical hypothyroidism and in hyperthyroidism were not different from those of the euthyroid subjects.

The differences between the groups described for HL and LPL activities and for the lipoprotein concentrations were significant also when the values of the women were calculated separately to eliminate the possible influence of different sex distribution in the groups.

Table 2 summarizes the correlations between S-T₃ concentrations, HL and LPL activities, and lipoprotein concentrations calculated for the individuals in all groups together. Of the various transformations tested the logarithmic transformation was found to give the best adaptation to a rectilinear relationship throughout.

Very high correlations were found between S-T₃ and S-T₄ ($r = 0.95$, $P < 0.001$) and between S-T₃ and FTI ($r = 0.97$, $P < 0.001$). S-T₃ was found to give slightly better correlations to lipoprotein concentrations and enzyme activities than S-TSH, S-T₄ and FTI.

The total and LDL cholesterol concentrations were strongly correlated ($r = 0.98$, $P < 0.001$). The coefficient of correlation between S-T₃ and LDL cholesterol increased from -0.60 to -0.76 after logarithmic transformation of S-T₃ (Fig. 1). The correlations between S-T₃ or $\log$ S-T₃ and HDL cholesterol were weak or insignificant (Table 2). The coefficient of correlation for S-T₃ vs P-triglyceride was -0.17 , increasing to -0.37 ($P < 0.01$) after logarithmic transformation of S-T₃.

A highly significant correlation was found between S-T₃ and HL activity ($r = 0.77$, $P < 0.001$).

Table 1.

Thyroid function variables, hepatic lipase and lipoprotein lipase activities, lipoprotein concentrations and the iv fat tolerance test in 70 individuals, allocated into 4 groups according to thyroid function. Data are given as mean $\pm$ SEM. Reference ranges are those routinely used in our laboratory. LDL cholesterol concentrations were calculated according to Friedwald et al. (1972) and include IDL cholesterol.

	Mean age (years)	Mean weight (kg)	S-T ₃ (nmol/l)	FTI	TSH (mU/l)	HL activity (mU/ml)	LPL activity (mU/ml)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	TG (mmol/l)	IVFTT (%/min)
Group 1 (n = 20); 7 men, 13 women											
Overt											
hypothyroidism	47.6	74.8	0.46 $\pm$ 0.04	17.2 $\pm$ 0.9	122.2 $\pm$ 23.4	204.3 $\pm$ 21.1	75.6 $\pm$ 9.0	7.09 $\pm$ 0.55	1.22 $\pm$ 0.09	2.15 $\pm$ 0.43	2.76 $\pm$ 0.20
P vs group 2			< 0.001	< 0.001	< 0.025	n.s.	< 0.05	< 0.001	n.s.	< 0.025	< 0.001
P vs group 3			< 0.001	< 0.001	< 0.001	< 0.005	< 0.001	< 0.001	n.s.	< 0.01	
Group 2 (n = 12); 2 men, 10 women											
Subclinical											
hypothyroidism	49.9	64.7	1.53 $\pm$ 0.15	46.6 $\pm$ 5.6	61.3 $\pm$ 8.9	313.3 $\pm$ 53.2	109.2 $\pm$ 12.5	4.63 $\pm$ 0.26	1.39 $\pm$ 0.07	0.92 $\pm$ 0.13	6.12 $\pm$ 0.83
P vs group 3			< 0.05	< 0.001	< 0.001	n.s.	n.s.	< 0.001	n.s.	n.s.	
Group 3 (n = 17); 9 men, 8 women											
Euthyroidism	34.7	75.5	1.93 $\pm$ 0.09	93.1 $\pm$ 3.6	3.1 $\pm$ 0.2	349.9 $\pm$ 40.9	130.4 $\pm$ 11.2	3.46 $\pm$ 0.18	1.26 $\pm$ 0.08	0.90 $\pm$ 0.90	—
Group 4 (n = 21); 5 men, 16 women											
Hyperthyroidism	40.9	59.6	7.99 $\pm$ 0.71	445.3 $\pm$ 33.8	1.1 $\pm$ 0.1	659.7 $\pm$ 47.7	121.7 $\pm$ 6.8	2.52 $\pm$ 0.17	1.04 $\pm$ 0.06	1.10 $\pm$ 0.09	6.48 $\pm$ 0.51
P vs group 3			< 0.001	< 0.001	< 0.001	< 0.001	n.s.	< 0.001	< 0.05	n.s.	
Reference range			0.9–3.2	0–8	0–8	220–520	110–220	1.7–4.4	0.8–1.6	0.4–2.1	5.0–6.9

Table 2.

Coefficients of correlation between S-T₃, lipoprotein concentrations and the activities of hepatic lipase and lipoprotein lipase in 70 individuals with thyroid function ranging from overt hypothyroidism to hyperthyroidism.

	Plasma cholesterol	LDL cholesterol	HDL cholesterol	Plasma triglyceride	HL	LPL
S-T ₃	-0.61 a	-0.60 a	-0.26 c	-0.17*	+0.77 a	+0.28 c
log S-T ₃	-0.79 a	-0.76 a	-0.18*	-0.37 b	+0.76 a	+0.45 a
HL	-0.55 a	-0.52 a	-0.35 b	-0.12*		
log HL	-0.59 a	-0.55 a	-0.39 a	-0.17*		
LPL	-0.46 a	-0.43 a	+0.21*	-0.49 a		
log LPL	-0.55 a	-0.48 a	+0.22*	-0.71 a		

a: $P < 0.001$; b: $P < 0.01$; c: $P < 0.05$; * not significant.

(Fig. 2) without improvement after logarithmic transformation. The correlation between S-T₃ and LPL activity was weaker and evident only after logarithmic transformation of S-T₃ (Table 2).

Total plasma and LDL cholesterol levels were both strongly correlated to HL but more weakly to LPL without significant improvement after transformation (Table 2). When related to HDL cho-

lesterol the coefficients of correlation were lower for both enzymes; however, the relationship between HDL cholesterol and HL activity was significant (Table 2). As expected, P-triglyceride concentrations were significantly correlated to the LPL activity, the coefficient of correlation increasing from -0.49 to -0.71 after logarithmic transformation of LPL values.

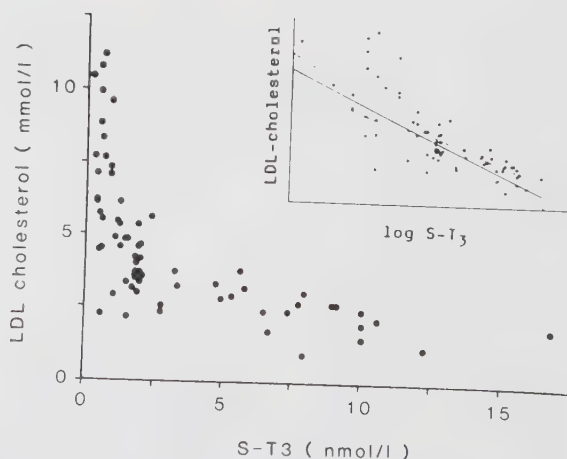


Fig. 1.

Relationship between S-T₃ and LDL cholesterol concentrations in 70 subjects with different levels of thyroid function. In semilogarithmic representation the correlation coefficient was -0.76 ($P < 0.001$). The broken lines indicate the 95% confidence limits for the regression line.

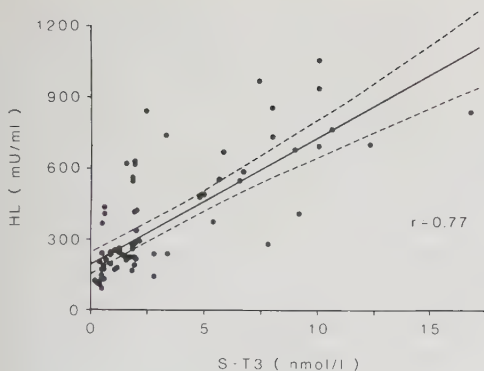


Fig. 2.

Relationship between S-T₃ concentrations and hepatic lipase activities in 70 subjects with different levels of thyroid function. Correlation coefficient: 0.77 ($P < 0.001$). The broken lines indicate the 95% confidence limits for the regression line.

Discussion

Thyroid function

Hypothyroidism is a graded phenomenon and can be subdivided into an overt form with clinical myxoedema and subclinical forms with no or only mild clinical symptoms. The laboratory parameter that best reflects thyroid hormone action seems to be S-T₃ (Chopra et al. 1978; Bantle et al. 1980). A lowered S-T₃ as a line of demarcation between overt and subclinical hypothyroidism has been suggested by Bastenie (1980). Therefore we adopted the lower reference limit for our laboratory for S-T₃ as an arbitrary line of division between overt and subclinical hypothyroidism, and used the upper reference limit for S-TSH to differentiate the hypothyroid groups from normals, and an elevated S-T₃ as the criterion of hyperthyroidism.

The mean values in our grouped material were registered to permit comparisons with other materials. However, they suggested continuous changes with thyroid function of certain lipoprotein classes as well as of lipase activities over the entire range of thyroid function. The associations between these variables were therefore further elucidated by correlations of individual values for all participating subjects. In most cases the relationship between hormone concentrations and lipase activities or lipoprotein levels conformed better to the straight

line after logarithmic transformation of hormone values. This is in accordance with the characteristically rectilinear relation seen between log concentrations and effects of most hormones. S-T₃ generally proved to be slightly stronger correlated to lipoprotein concentrations and lipase activities than S-T₄, FTI or S-TSH. This is consistent with the concept of triiodothyronine as the principal mediator of thyroid hormone action (Chopra et al. 1978).

Thyroid function and lipoprotein levels

The lipoprotein profiles of our patient groups are, in general, in agreement with earlier studies in more limited groups of patients with thyroid dysfunction (Walton et al. 1965; Rössner & Rosenqvist 1974; Agdeppa et al. 1979). It should be pointed out that the measure of LDL cholesterol (Friedewald et al. 1972) used in this study will also include intermediate density lipoprotein (IDL) cholesterol. Most prominently, plasma LDL concentrations showed a continuous spectrum from elevated levels in severe hypothyroidism to concentrations in the lower reference range in hyperthyroidism. Correlations between individual values of S-T₃ and LDL cholesterol demonstrated a curvi-linear relation with a better adaptation to a straight line after logarithmic transformation of S-T₃. A similar relationship has been demonstrated between S-T₃ and total plasma cholesterol values (Ikejiri et al. 1978; Bantle et al. 1980).

Thyroid function and lipase activities

Lipoprotein lipase catalyzes the rate-limiting step in triglyceride removal from VLDL and chylomicrons, a process that is also associated with the formation of HDL cholesterol whereas hepatic lipase seems involved in the removal of lipoprotein surface components (especially phospholipid and cholesterol), probably acting as a phospholipase using HDL as its substrate (Nilsson-Ehle et al. 1980). AS pointed out above both HL and LPL seem to be of significance for the dyslipoproteinaemia in hypothyroidism whereas the role of these enzymes in hyperthyroidism is less well-known.

In a group of hypothyroid patients comprising overt as well as subclinical forms, LPL activity did not change significantly upon treatment with thyroxine (Abrams et al. 1981). However, our present results confirm a report indicating that LPL activity is low in overt but not in subclinical hypothyroidism (Lithell et al. 1981). Thus a separation of these

forms of hypothyroidism seems necessary to detect an association between thyroid hypofunction and LPL activity. Our hyperthyroid patients did not differ from euthyroid subjects in LPL activity, which is consistent with the unchanged activity found after treatment for hyperthyroidism (Abrams et al. 1981). This pattern together with the weak correlation between S-T₃ and LPL values suggests an indirect effect of T₃ on LPL activity e.g. via an effect on protein synthesis (Tata & Widnell 1966). Such a mechanism is also consistent with the delayed effect of thyroid hormone treatment on LPL activity in hypothyroidism (Valdemarsson et al. 1982).

In contrast, HL activity was related to S-T₃ concentrations over the full spectrum of thyroid function, with a 3-fold difference between patients with hypo- and hyperthyroidism. The group mean value was significantly elevated in hyperthyroidism. A differently designed study by Abrams et al. (1981) and Abrams & Grundy (1981) showed only insignificant reduction of HL activity upon treatment for hyperthyroidism. However, a cross-sectional design with a euthyroid reference group may be more powerful in demonstrating an elevated HL activity in hyperthyroidism. The strong correlation between S-T₃ and HL values supports the view that T₃ has a direct effect on HL regulation (Hansson et al., in press). This is consistent with the rapid response of HL activity to T₃ replacement in hypothyroidism (Valdemarsson et al. 1982).

Interrelations between hormone, enzyme and lipoprotein levels

The metabolism of triglyceride-rich lipoproteins seems severely impaired only in overt hypothyroidism. These patients had elevated P-triglyceride levels, decreased LPL activities and impaired elimination of exogenous triglyceride. These variables were all statistically intercorrelated, and also correlated to S-T₃ levels. The correlation coefficient between log LPL and P-TG concentration was -0.71 , corresponding to a coefficient of determination of 0.50 ; i.e., 50% of the variation in P-TG can be explained by differences in LPL activity. When S-T₃ was introduced as second variable in a multiple regression analysis, the coefficient of determination did not increase, demonstrating that S-T₃ was not correlated to P-triglyceride concentrations independently of LPL. These data support the view that LPL is a strong determinant of plasma

triglyceride levels. The impact of T₃ on P-TG levels seems to be exerted mainly via the LPL mechanism.

Another group of correlations emerges around the metabolism of LDL. When hypothyroid patients were treated, the changes in LDL cholesterol concentration, S-T₃ levels, and HL activities were intercorrelated (Valdemarsson et al. 1982). In the present investigation S-T₃ levels, HL activities and LDL cholesterol concentrations were all intercorrelated over the full spectrum of thyroid function states. In this case, the independence of the two possible determinants of LDL cholesterol, i.e., S-T₃ and HL activity, is difficult to establish by statistical methods. The coefficients of determination did not increase significantly when both S-T₃ and HL activity were included in the multiple regression analysis, a fact that is readily explained by the high correlation ($r = 0.77$) between these two determinants. HL is considered to act upon HDL as its direct substrate (Nilsson-Ehle et al. 1980). It is possible that HL, under direct influence of T₃ enhances the elimination of cholesterol by facilitating the flow of surface components from VLDL-LDL via HDL to the liver. However, the strong correlation between log S-T₃ and plasma LDL levels ($r = -0.76$) indicates an additional mode of operation for T₃ in LDL regulation, such as a direct effect of T₃ on the LDL receptor (Chait et al. 1979).

Acknowledgments

Ms Gerd Nilsson, Ms Lilly Berlin, Ms Britt Englesson and Brita Sundén-Andersson gave excellent technical assistance. We are grateful to Associate Professor Claus Rerup for assistance with the computer programmes and to Jerker Ringström and Lars Skareke for statistical assistance. Financial support was obtained from the Swedish Medical Research Council (04966), Pålssons Foundation, Malmö and the Medical Faculty, University of Lund.

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Received on September 7th, 1982.

Experimental Hyperthyroidism in Man: Effects on Plasma Lipoproteins, Lipoprotein Lipase and Hepatic Lipase

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Summary

We have studied the effects of triiodothyronine administration (20–40 µg three times daily over one week) in six healthy young men, on the activities of lipoprotein lipase and hepatic lipase and on plasma lipoprotein concentrations. Hepatic lipase activity in post-heparin plasma rose by $46 \pm 25\%$ ($p < 0.025$), whereas the activity of lipoprotein lipase did not change significantly. Plasma cholesterol concentrations decreased by about 20% ($p < 0.025$), whereas there was no change in plasma triglyceride levels. The fall in plasma cholesterol could be accounted for by a reduction of HDL cholesterol (-11%, $p < 0.025$) as well as LDL cholesterol (-27%, $p < 0.025$). The data emphasize the role of hepatic lipase in the lipoprotein alterations associated with thyroid dysfunction.

Key-Words: Hepatic Lipase – Hyperthyroidism – Lipoproteins – HDL – Lipoproteins – LDL – Lipoprotein Lipase – Triiodothyronine

Introduction

Thyroid dysfunction is associated with alterations in the composition and concentrations of all major lipoprotein classes (Walton, Scott, Dykes and Davies 1965; Rössner and Rosenqvist 1974; Ballantyne, Epenetos, Caslake, Forsythe and Ballantyne 1979; Scottolini, Bhagavan, Oshiro and Abe 1980; Agdeppa, Macaron, Mallik and Schnuda 1979). The mechanisms responsible for the various dyslipoproteinemias are still incompletely understood. Besides the rate of production and elimination, the pattern of intravascular metabolism also influences the concentrations of plasma lipoproteins.

Recent studies indicate that alterations in the activities of lipoprotein lipase (LPL) and hepatic lipase (HL), two key enzymes in plasma lipoprotein metabolism, are important factors in the pathogenesis of dyslipoproteinemia seen in thyroid disease. Thus, LPL activity in adipose tissue (Pykalistö, Goldberg and Brunzell 1976; Lithell, Broberg, Helsing, Ljunghall, Lundqvist, Vessby and Wide 1981), skeletal muscle (Lithell et al. 1981) and post-heparin plasma (Lithell et al. 1981; Valdemarsson, Hedner and Nilsson-Ehle 1982) is low in severely hypothyroid patients and increases on treatment. Mild thyroid hypofunction, however, does not seem to affect LPL activities (Lithell et al. 1981; Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981). Studies on LPL activity in hyperthyroidism have

yielded conflicting results (Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981; Arons, Schreiber, Downs, Braverman and Arky 1972; Kirkeby 1968; Tulloch, Lewis and Fraser 1973; Nikkilä and Kekki 1972).

The effects of thyroid disease on HL activity have been less extensively studied. The enzyme activity is low in hypothyroidism (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981; Krauss, Levy and Fredrickson 1974), and substitution therapy rapidly restores HL activity to normal levels (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981). A recent study indicates that HL activities may be slightly elevated in hyperthyroidism (Abrams, Grundy and Ginsberg 1981).

In the present study we have investigated the time-course for the effects of experimental hyperthyroidism, induced by administration of triiodothyronine (T_3) to healthy subjects, on the activities of LPL and HL, and on plasma lipoprotein concentrations.

Materials and Methods

Experimental design

The experiment was approved by the local Ethics' Committee. Six men, aged 20–36 years, volunteered. They were all euthyroid and had no history or signs of endocrine or metabolic diseases. On Day 0, peroral administration of T_3 (Nyegaard A/S, Oslo, Norway), 20 µg three times daily, was started. On Day 2 the dose was doubled and the medication continued through day 6. Blood samples were obtained at 8 a.m. on days -5, -3, 1, 2, 4 and 7, the subjects fasting for at least 8 h. The initial values represent the mean of data obtained from the two samples collected prior to T_3 administration. Blood was drawn for the determination of T_3 , thyroxine (T_4), thyrotropin (TSH) and the T_3 uptake test, plasma cholesterol, HDL cholesterol and triglycerides. Fifteen minutes after the intravenous injection of heparin (50 U/kg body weight) blood was drawn for determination of LPL and HL activities in post-heparin plasma (Nilsson-Ehle and Ekman 1977). Separate control experiments demonstrated that the repeated heparin administration as such did not affect the lipoprotein concentrations or lipase activities.

Analytical Methods

T_3 and TSH concentrations were determined by commercial radioimmunoassay kits (Skandinaviska Immunlaboratoriet AB, Malmö, Sweden). T_4 was determined by the method of Cheung and Stanwhite (1976), using anti- T_4 -antiserum from Farnos Diagnostica, Turku, Finland.

The T_3 uptake test was performed according to Nosslin (1965).

Cholesterol and triglyceride were determined by enzymatic methods (Boehringer Mannheim GmbH, West Germany). HDL cholesterol

Table 1 Laboratory tests reflecting thyroid function in six healthy subjects during triiodothyronine administration. Values are given as mean $\pm$ S.D. Asterisks indicate significances of differences compared to initial values (* $p < 0.05$; ** $p < 0.025$). Reference ranges are those routinely used in our laboratory.

	Initial value (mean of two analyses in each person)	Day 1	Day 2	Day 4	Day 7	Reference range
T_3 (nmol/l)	1.82 ± 0.37	3.67 ± 0.74 **	3.82 ± 0.88 **	6.28 ± 2.04 **	5.88 ± 1.29 **	0.9 – 3.2
T_4 (nmol/l)	85.2 ± 11.6	88.2 ± 11.9	67.0 ± 14.9 *	62.2 ± 15.2 **	57.3 ± 13.3 **	57 – 154
TSH (mIU/l)	2.6 ± 1.5	2.1 ± 0.9	1.8 ± 0.9	2.5 ± 0.6	2.0 ± 0.5	< 8
T_3 uptake (arbitrary units)	1.12 ± 0.14	0.99 ± 0.09 *	0.94 ± 0.08 *	1.00 ± 0.07 **	0.89 ± 0.11 **	0.80 – 1.20

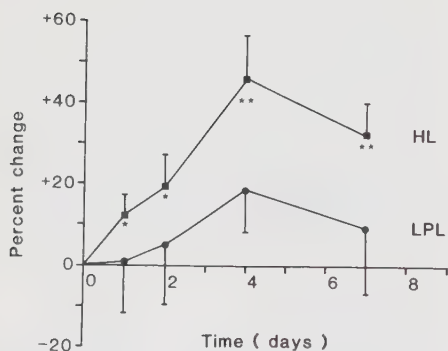


Fig. 1 Time-course of changes in activities of hepatic lipase (HL) and lipoprotein lipase (LPL). The initial enzymatic activities (mean $\pm$ S.D.) were 506 ± 161 mU/ml plasma (HL), and 136 ± 38 mU/ml (LPL), respectively; one mU represents the release of one nmol fatty acid per minute. Bars represent SEM. * $p < 0.05$, ** $p < 0.025$.

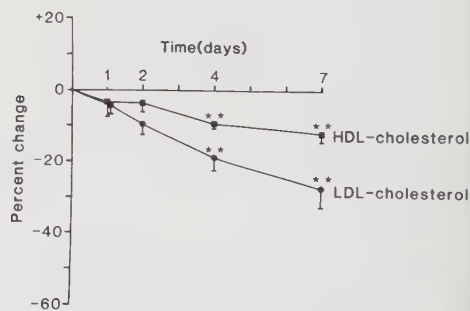


Fig. 2 Relative changes in plasma HDL cholesterol and LDL cholesterol levels during triiodothyronine intake in healthy men. Initial concentrations were 1.12 ± 0.36 mmol/l and 3.40 ± 0.52 mmol/l (mean $\pm$ S.D.), respectively. Bars represent S.E.M. ** $p < 0.025$.

was measured after precipitation of VLDL and LDL by dextran sulphate and $MgCl_2$ (Danielsson, Ekman, Fax, Johansson, Kristensson, Nilsson-Ehle and Wadstein 1978).

LDL cholesterol was calculated by the formula: LDL cholesterol = Total cholesterol – (HDL cholesterol $\times$ Triglycerides $\times$ 0.45) (Modified to SI Units from Friedewald, Levy and Fredrickson 1972). LPL and HL activities were determined by specific methods described earlier (Nilsson-Ehle and Ekman 1977), using dioleoyl phosphatidyl choline (final conc 0.067 mg/ml) instead of lyso-phosphatidyl choline as substrate emulsifier.

Statistics

The significance of differences was evaluated by Wilcoxon's rank order test for the paired case. Correlations between variables were evaluated by linear regression analysis.

Results

On administration of T_3 five of the subjects reported symptoms of hyperthyroidism. Serum T_3 levels increased to values above the upper reference limit in all subjects (Table 1). The concentrations of TSH remained unchanged, whereas serum T_4 levels decreased continuously during the experiment (Table 1). There was also a decrease in the T_3 uptake test.

HL activities increased rapidly in all subjects, roughly parallel to the rise in serum T_3 concentrations, to levels $46 \pm 25\%$ ($p < 0.025$) above the initial (Fig. 1). Five of the 6 subjects reached enzyme activities above the upper reference limit. In contrast, the moderate rise in LPL activities (Fig. 1) did not reach statistical significance.

The concentrations of plasma cholesterol, HDL cholesterol, and LDL cholesterol all gradually decreased (Fig. 2). The differences noted on day 4 and 7 reached statistical significance. The most pronounced change was recorded for LDL cholesterol (-27%, $p < 0.025$). The decrease in HDL cholesterol averaged 12% ($p < 0.025$). The decrease in HDL cholesterol was equally pronounced in the subjects with initial values in the lower and upper reference range. No significant change was noted for the concentrations of plasma triglycerides.

There were no correlations between individual values for serum T₃, HL activity or lipoprotein concentrations nor for the changes in these variables.

Discussion and Conclusions

In the present experiment we chose to induce experimental hyperthyroidism by administration of T₃, which is the biologically more active thyroid hormone (Thorell and Larsson 1978; Chopra, Solomon, Chopra, Sing Yung, Fisher and Nakamura 1978). The rise in T₃ levels was accompanied by a fall in T₄ concentrations, indicating suppression of the endogenous T₄ production. The limited sensitivity of the TSH radioimmunoassay (Thorell and Larsson 1978) probably explains why we could not detect a fall in TSH concentrations. The decreasing values of the T₃ uptake test are probably secondary to the decrease in T₄ levels, since the thyroid-hormone-binding proteins of plasma have a higher affinity for T₄ than for T₃ (Thorell and Larsson 1978).

Previous studies have shown that HL activity is low in hypothyroidism (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsburg 1981; Krauss, Levy and Fredrickson 1972) and rises on substitution therapy (Valdemarsson, Hedner and Nilsson-Ehle 1982; Abrams, Grundy and Ginsberg 1981). This study extends these observations, demonstrating that HL activity in healthy subjects can be raised above the upper reference limit in experimental hyperthyroidism. The rise in enzyme activity was roughly parallel to the increase in T₃ concentrations. These data strongly suggest that T₃ plays a direct role in the physiological regulation of HL activity, which is consistent with earlier studies demonstrating a relationship between the increase in S-T₃ levels and HL activities recorded after substitution of hypothyroid patients (Valdemarsson, Hedner and Nilsson-Ehle 1982). Thus it seems that alterations in HL activity accompany both hyper- and hypothyroidism (Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981).

The role of thyroid hormones in the regulation of LPL activity seems less clear-cut. Earlier studies indicate that this enzyme activity is low only in overt hypothyroidism and normalizes on treatment (Pykalisto, Goldberg and Brunzell 1976; Lithell et al. 1981; Abrams, Grundy and Ginsberg 1981; Abrams and Grundy 1981; Valdemarsson, Hedner and Nilsson-Ehle 1982). In subclinical thyroid hypofunction the enzyme activity seems essentially normal (Lithell et al. 1981). The present study demonstrates that the LPL activity responds poorly to an elevation of serum

T₃ concentration, and despite clinical and laboratory signs of hyperthyroidism, our subjects showed no significant increase in LPL activity. Thus it seems that adequate concentrations of thyroid hormones are necessary for the maintenance of normal LPL activity.

However, the iodothyronines seem to have a less prominent role in the short-term physiological regulation of LPL activities. This view is consistent with our suggestion (Valdemarsson, Hedner and Nilsson-Ehle 1982) that the effect of T₃ on LPL activity is mainly exerted through its positive effect on protein synthesis (Tata and Widnell 1966).

The observed decrease in HDL cholesterol levels is consistent with studies in hyperthyroid patients, who generally have HDL cholesterol levels in the lower reference range (Agdeppa et al. 1979; Scottolini et al. 1980).

Agdeppa et al. (1979) studied the effects of T₄ administration (0.3 mg daily for one month) on HDL cholesterol concentrations in normal subjects, but could detect no change. The apparent discrepancy with our study may be explained by the different iodothyronines used. Their T₄ dose may have been too low to maintain sufficient levels of biologically active T₃ during their four weeks study (Thorell and Larsson 1978; Chopra et al. 1978).

The decrease in HDL cholesterol, parallel with the selective increase in HL activity, supports present views of HL as an enzyme involved in the degradation of HDL (Shirai, Barnhart and Jackson 1981), probably acting as a phospholipase using HDL₂ as substrate. We did not quantitate the subfractions HDL₂ and HDL₃ separately in this study. However, extrapolation from another study (Brook, Aviram, Luboshitzky and Barzilai 1981) would indicate that the decrease was confined mainly to the HDL₂ subfraction.

LDL cholesterol concentrations decreased, as could be expected from earlier studies in hyperthyroid patients (Scottolini et al. 1980) as well as in normal subjects receiving an excess of T₄ (Agdeppa et al. 1979). The mechanisms may include both an increased number of LDL receptors (Chait, Bierman and Albers 1979) and an enhanced transfer of cholesterol and phospholipid to the HDL subfraction with subsequent rapid uptake in the liver via HL (Valdemarsson, Hedner and Nilsson-Ehle 1982).

Acknowledgements

Britt Engleson, Gerd Nilsson and Brita Sundén-Andersson gave skilful technical assistance. This project was sponsored by the Medical Faculty, University of Lund, the Swedish Medical Research Council (04966) and Pahlssons Foundation, Malmö.

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BBA 51489

INFLUENCE OF NUTRITIONAL STATE ON LIPOPROTEIN LIPASE ACTIVITIES IN THE HYPOTHYROID RAT

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(Received March 14th, 1983)

Key words: Lipoprotein lipase; Hypothyroidism; Nutritional state; (Rat)

We have investigated the effects of nutritional state on the lipoprotein lipase activities of the experimentally hypothyroid rat. Both short-term effects (i.e., those of a 24 h fast with and without re-feeding) and long-term effects (due to decreased food intake in hypothyroidism) have been studied. The hypothyroid rats had significantly higher lipoprotein lipase activities of adipose tissue and heart muscle. The effect of hypothyroidism on adipose tissue lipoprotein lipase activities was modified by the nutritional state. In rats studied after 24 h fasting, the hypothyroid group had significantly higher lipoprotein lipase activities than weight-matched, age-matched and pair-fed (i.e., semi-starved) control groups. In rats studied in the re-fed state, the effects of hypothyroidism as such were less evident, since the pair-fed group also demonstrated significantly higher enzyme activities than did the other control groups. We have also studied the lipoprotein lipase activities of different enzyme preparations from adipose tissue. The effects of hypothyroidism were most clearly reflected in an increase of heparin-elutable enzyme activity from adipose tissue, whereas adipocyte lipoprotein lipase activity and the lipoprotein lipase secretion rate from adipocytes were affected to a lesser extent. We conclude that alterations in food intake strongly influence the lipoprotein lipase activities in the hypothyroidism. Our data also imply that the increased lipoprotein lipase activity in the hypothyroid state is due to a decreased degradation of the enzyme, both intra- and extracellularly.

Introduction

Hypothyroidism is accompanied by severe abnormalities in lipoprotein metabolism. One of the factors contributing to the pathogenesis of the dyslipoproteinemia is an altered activity of lipoprotein lipase (EC 3.1.1.34). However, comparison of data from man and from the rat demonstrates obvious qualitative and quantitative species dif-

ferences with regard to thyroid function and lipoprotein lipase activity.

In man, overt hypothyroidism is accompanied by a decreased lipoprotein lipase activity in adipose tissue [1,2], skeletal muscle [2] and post-heparin plasma; the activity rises on hormonal substitution [1–3]. In contrast, experimental hypothyroidism in the rat leads to an increase in lipoprotein lipase activity of adipose tissue [4–9] whereas the enzyme activity in heart has been reported to be normal [10] or decreased [5].

In this study we wanted to investigate further the altered activity of lipoprotein lipase in the hypothyroid rat and its relation to nutritional state. Hypothyroidism in the rat drastically reduces food intake and weight gain [11], factors that in them-

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Abbreviations: PTU, 6-*n*-propyl-2-thiouracil HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins; TSH, thyrotropin; T₃, 3,5,3'-triiodothyronine; rT₃, 3,3',5'-triiodothyronine; T₄, thyroxine.

selves affect lipoprotein lipase activity [12]. Therefore, we included a group of euthyroid pair-fed rats in the experiment, as well as rats with free access to food. In addition, the short-term effects of nutritional state were investigated by comparison of fasted and re-fed rats.

To gain further knowledge of the mechanisms involved in the altered lipoprotein lipase regulation, we measured lipoprotein lipase activities of heart and adipose tissues representing different pools of enzyme activity.

Experimental procedures

Experimental design

Male Sprague-Dawley rats (Anticimex AB, Stockholm) were used. The animals were maintained in single cages at a temperature of 25°C at a 6 a.m. to 6 p.m. light cycle. They were fed Astra-Ewos R3 chow (Astro-Ewos AB, Södertälje, Sweden), which contains 28% of the energy as protein, 13% as fat and 58% as carbohydrate.

Hypothyroidism was induced by 6-*n*-propyl-2-thiouracil (PTU), added to the drinking water at a concentration of 1 g/l. Three groups of control rats were used: one group (-PF) was individually pair-fed with the hypothyroid rats (-PTU); one had free access to food (age-matched, -AM); and one group of weight-matched animals (-WM) was selected among younger rats also having free access to food.

Animals were killed after 13–23 days on the above regimens. Their food was withdrawn 24 h before killing. Animals assigned to be re-fed (R-) were given 5 g of chow at 7.30 a.m. and were killed by decapitation 2 h later. Other rats (F-) were killed at 8 a.m. while still fasting.

Blood was collected in glass tubes, and serum prepared. Retroperitoneal adipose tissue and heart were removed and immediately frozen at -70°C. Epididymal adipose tissue was excised and processed directly.

64 rats were used in the study, namely eight rats of each of the following groups: re-fed, hypothyroid (R-PTU), re-fed, pair-fed (R-PF), re-fed age and weight matches (R-AM, R-WM), fasted, hypothyroid (F-PTU), fasted, pair-fed (F-PF) and fasted age and weight matches (F-AM, F-WM).

Measurements of lipoprotein lipase activities in heart and adipose tissues

To determine heparin-elutable lipoprotein lipase activity, epididymal adipose tissue (about 20 mg) was coarsely cut and incubated in a Krebs-Ringer/ HCO_3^- /buffer (pH 7.4) containing 6 units/ml of heparin [13]; after 20 min the enzyme activity eluted into medium was determined. The remaining tissue was extracted by isopropanol/heptane/0.5 M H_2SO_4 (40:10:1, by vol.); after partition against an acidified aqueous phase the lipid weight was determined. These enzymatic activities were expressed relative to the lipid weight of the sample.

Retroperitoneal adipose tissue and heart were taken from refrigeration and immediately extracted by cold acetone and diethyl ether (25 and 10 times by vol., respectively) [14]. The resulting powders were solubilized in 0.1 M Tris-HCl buffer, pH 7.4, containing 1 M ethylene glycol [15]. After centrifugation, the lipoprotein lipase activity in the clear supernate was assayed and related to the protein content of the solution [16]. The same procedure was used to measure the enzyme activity in heart.

Measurements of adipocyte lipoprotein lipase

Isolated adipocytes were prepared from epididymal adipose tissue by collagenase treatment [17]. Insulin and glucose were excluded from the solutions when preparing cells from fasted rats, since insulin may then rapidly increase lipoprotein lipase activity of adipose tissue from fasted rats [18]. The mean cell size was estimated by microscopy of 100 cells in each preparation.

Cellular lipoprotein lipase activity was measured in acetone/diethyl ether powders prepared from isolated adipocytes. The cells were extracted by cold acetone and diethyl ether in glass homogenators [19]. Due to the low protein content of adipocytes, bovine serum albumin was added to produce sufficient protein precipitate. Tris-HCl buffer with 1 M ethylene glycol was used to solubilize the powders. The lipid weights of the samples were determined from the lipid extract.

Isolated adipocytes were also used to measure lipoprotein lipase secretion [15,20]. 20 μl of human heat-inactivated serum was added to 500 μl of an adipocyte suspension (1:4) in Krebs-Ringer/ HCO_3^- buffer. After a 30-min incubation at 37°C

during slow shaking, adipocytes were floated by rapid centrifugation and the infranant medium collected and assayed for lipoprotein lipase activity.

Analytical methods

Lipoprotein lipase activity was assayed by the method of Nilsson-Ehle and Ekman [21], using phosphatidylcholine (final concentration, 0.067 mg/ml) as substrate emulsifier. Commercial radioimmunoassay kits were used to determine serum concentrations of 3,5,3'-triiodothyronine (T_3) (SILAB, Malmö, Sweden), thyroxine (T_4) (Farnos Diagnostica, Turku, Finland) and 3,3',5'-triiodothyronine (rT_3) (Serono-Biodata, Coinsins, Switzerland). Rat thyrotropin (TSH) was measured by a radioimmunoassay kit generously donated by NIAMDD (Dr. A.F. Parlow); purified TSH (RP-1) was used as standard. Enzymatic kits from Boehringer Mannheim GmbH were used for determination of serum triacylglycerols and cholesterol. The latter kit was also used to determine HDL cholesterol after precipitation of VLDL and LDL by dextran and $MgCl_2$ [22]. HDL phosphatidylcholine was estimated using a kit from Wako Biochemicals, Osaka, Japan.

Chemicals

PTU was purchased from Sigma Inc. It was dissolved in distilled water and kept in dark bottles before and during use. Fresh solutions were prepared each week. Dioleoylphosphatidylcholine and trioleoylglycerol were also purchased from Sigma Inc; the latter was purified by silicic acid chromatography before use. Tri[3H]oleoylglycerol was synthesized from [3H]oleic acid and glycerol and purified to a radiopurity of over 99.6% before use [19]. All other chemicals were analytical grade.

Results

The food intake of all rats tended to increase slightly after allocation to single cages. Hypothyroid animals ate about 40% less than ad libitum-fed controls, resulting in a considerably slower weight gain. At the time of killing the ad libitum-fed controls (R-, F-AM) were significantly heavier than all other groups, which was also reflected by significantly larger adipocytes (Fig. 1, Table I).

Hormonal data are summarized in Table II. PTU treatment resulted in an almost total disappearance of T_4 , and a marked reduction in T_3 concentrations. Despite the drastic reduction of T_4 , rT_3 levels were not affected. TSH concentrations increased markedly following PTU treatment. TSH levels were generally higher in the euthyroid animals killed in the refed state ($P < 0.05$).

Table III shows lipoprotein lipase activities. In general, measurements in acetone/diethyl ether powders and heparin eluates gave the same qualitative information, both methods reflecting the adipose tissue content of enzyme [13]. In the fasted animals, a drastic increase in the enzyme activity of adipose tissue was seen in hypothyroidism (F-PTU). Body weight (F-AM) or long-term food restriction (pair-feeding) (F-PF) did not affect lipoprotein lipase activities in the fasted rats. The adipocyte lipoprotein lipase activity was depressed

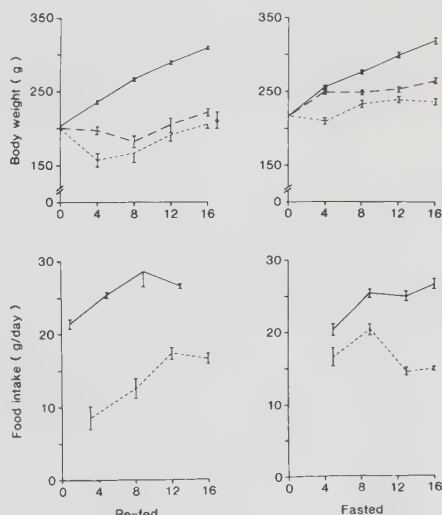


Fig. 1. Body weights and food intakes of age-matched (—), pair-fed (---) and hypothyroid rats (···) examined in the re-fed (left) and fasted (right) states. Data are given as mean $\pm$ S.E. Pair-feeding was started on day 0 in the 're-fed' group, and on day 4 in the 'fasted' group. The solitary symbols to the right of body weight diagrams indicate the weights of the weight matched rats at time of killing.

TABLE I

BODY WEIGHT, FOOD INTAKE AND ADIPOCYTE SIZE OF HYPOTHYROID RATS AND CONTROL GROUPS

Each group consists of eight rats. Data are given as mean $\pm$ S.E.

Group	Weight at death (g)	Food intake (g $\cdot$ day $^{-1}$) 2 days before killing	Adipocyte size (μ g lipid $\cdot$ cell $^{-1}$)
Re-fed			
Hypothyroid, R-PTU	205.6 $\pm$ 6.0	16.6 $\pm$ 0.7	0.076 $\pm$ 0.005
Pair-fed, R-PF	222.0 $\pm$ 5.2		0.073 $\pm$ 0.007
Age matched, R-AM	310.4 $\pm$ 2.1	26.6 $\pm$ 0.3	0.115 $\pm$ 0.006 **
Weight-matched, R-WM	210.6 $\pm$ 10.8	n.d.	0.080 $\pm$ 0.005
Fasted			
Hypothyroid, F-PTU	236.4 $\pm$ 4.6	15.0 $\pm$ 0.2	0.098 $\pm$ 0.004
Pair-fed, F-PF	265.0 $\pm$ 3.7		0.095 $\pm$ 0.009
Age-matched, F-AM	318.6 $\pm$ 4.8	25.1 $\pm$ 0.7	0.144 $\pm$ 0.012 **
Weight-matched, F-WM	257.6 $\pm$ 4.1	n.d.	0.111 $\pm$ 0.004

** Significantly different ($P < 0.01$) from other groups. n.d., not determined.

by the prolonged food restriction in the pair-fed group (F-PF).

This effect of food restriction was, however, abolished by the PTU treatment and there was no difference between the hypothyroid rats and the weight-matched controls (F-PTU vs. F-WM) (Table III). The serum stimulated lipoprotein lipase

secretion followed a pattern similar to that of the adipocyte enzyme activity.

Re-feeding, as expected, induced an increased lipoprotein lipase activity of adipose tissue, adipocytes and adipocyte lipoprotein lipase secretion; the differences were less pronounced in the heavier age matches (R-AM). As in the fasted

TABLE II

THYROID HORMONES AND TSH VALUES IN HYPOTHYROID RATS AND CONTROL GROUPS

The sensitivity of the T_4 radioimmunoassay was 11 nmol $\cdot$ l $^{-1}$. Data represent mean $\pm$ S.E. of eight animals, unless otherwise indicated. T_3 values below 0.5 nmol $\cdot$ l $^{-1}$ (the lower discrimination limit of the assay) have been assigned a value of 0.4 to allow statistical calculations. n.d., not detectable.

Group	T_3 (nmol $\cdot$ l $^{-1}$)	T_4 (nmol $\cdot$ l $^{-1}$)	rT_3 (nmol $\cdot$ l $^{-1}$)	TSH (ng $\cdot$ ml $^{-1}$)
Re-fed				
Hypothyroid, R-PTU	0.44 $\pm$ 0.02 ^b	n.d. ^c	0.60 $\pm$ 0.14	1289 $\pm$ 74 ^a
Pair-fed, R-PF	0.99 $\pm$ 0.06 ^a	57.2 $\pm$ 3.20	0.69 $\pm$ 0.12 ^a ($n = 7$)	201 $\pm$ 2 ($n = 7$)
Age-matched, R-AM	1.14 $\pm$ 0.03	59.51 $\pm$ 2.85	0.72 $\pm$ 0.13 ^a	424 $\pm$ 43
Weight-matched, R-WM	1.26 $\pm$ 0.06	61.43 $\pm$ 4.88	0.88 $\pm$ 0.12	501 $\pm$ 14 ($n = 6$)
Fasted				
Hypothyroid, R-PTU	0.4 ^c	n.d. ^c	0.46 $\pm$ 0.07 ($n = 6$)	1598 $\pm$ 101 ^c
Pair-fed, F-PF	0.87 $\pm$ 0.08	39.71 $\pm$ 3.67	0.77 $\pm$ 0.23 ($n = 4$)	186 $\pm$ 37 ($n = 4$)
Age-matched, F-AM	0.90 $\pm$ 0.04	50.75 $\pm$ 1.57 ^b	0.72 $\pm$ 0.07 ($n = 7$)	152 $\pm$ 31 ($n = 7$)
Weight-matched, F-WM	0.96 $\pm$ 0.06	42.14 $\pm$ 2.37	0.63 $\pm$ 0.06	267 $\pm$ 60

^a $P < 0.05$ compared to the respective weight-matched group.^b $P < 0.01$ compared to the respective weight-matched group.^c $P < 0.005$ compared to the respective weight-matched group.

TABLE III
LIPOPROTEIN LIPASE ACTIVITIES OF ADIPOSE TISSUE, ADIPOCYTES (INCLUDING LIPOPROTEIN LIPASE SECRETION RATE) AND HEART
Enzyme activities in adipose tissue were measured in acetone/diethyl ether powders (AE) after elution with heparin (HE). Data are given as mean $\pm$ S.E. of eight animals, unless otherwise indicated.

Group	Lipoprotein lipase activities		Adipocyte lipoprotein lipase secretion rate (munits $\cdot$ g $^{-1}$ lipid $\cdot$ 30 min $^{-1}$)	Heart lipoprotein lipase activities (munits $\cdot$ mg $^{-1}$ protein)
	Adipose tissue (AE) (munits $\cdot$ mg $^{-1}$ protein)	Adipose tissue (HE) (munits $\cdot$ g lipid)		
Re-fed	Hypothyroid, R-PTU	4.40 $\pm$ 1.24 ^a	251.26 $\pm$ 45.17 ^{c,d}	6.80 $\pm$ 2.36
	Pair-fed, R-PF	3.50 $\pm$ 0.78	143.44 $\pm$ 42.21 ^a	10.04 $\pm$ 1.87 ^a
	Age-matched, R-AM	0.79 $\pm$ 0.12 ^a	4.69 $\pm$ 1.48 ^c	0.94 $\pm$ 0.22 ^c
	Weight-matched, R-WM	1.69 $\pm$ 0.28	46.76 $\pm$ 18.23	3.61 $\pm$ 0.91
Fasted	Hypothyroid, F-PTU	1.84 $\pm$ 0.16 ^{b,e}	30.08 $\pm$ 10.19 ^{c,e}	3.22 $\pm$ 0.42 ^{c,e}
	Pair-fed, F-PF	0.73 $\pm$ 0.10	1.53 $\pm$ 0.21	1.27 $\pm$ 0.14 ^a
	Age-matched, F-AM	1.05 $\pm$ 0.08 ^a	1.74 $\pm$ 0.13 ^a	1.41 $\pm$ 0.19 ^a
	Weight-matched, F-WM	0.72 $\pm$ 0.17	2.18 $\pm$ 0.27	0.88 $\pm$ 0.11

^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.005$; compared to -WM. ^d $P < 0.05$, ^e $P < 0.005$; significant differences between -PTU and -PF.

animals, hypothyroidism was associated with a marked rise of lipoprotein lipase activity in adipose tissue. However, it seems that much of this elevation could be attributed to the lower food intake, since the enzyme activities in pair-fed rats were increased almost to the same extent (R-PTU vs. R-PF). The absolute differences between hypothyroid and pair-fed rats were larger if re-fed; however, the relative differences were much more pronounced in the fasting state (Table III). In the re-fed rats, with their higher cellular lipoprotein lipase activity, there was no additional effect of hypothyroidism. Similarly, no specific effects of hypothyroidism was noted on lipoprotein lipase secretion in the re-fed rats.

A marked increase in lipoprotein lipase activity of heart tissue was recorded in the hypothyroid animals (F-, R-PTU). Nutritional states and body weights influenced heart lipoprotein lipase activity only slightly in the euthyroid rats.

Plasma triacylglycerol concentrations tended to be lower in the hypothyroid rats than in the other groups, especially when fasted. However, the hypothyroid rats had significantly increased levels of plasma cholesterol (F-, R-PTU vs. F-, R-WM). As the changes in HDL cholesterol were small, the increase in cholesterol seems to be confined to the minor VLDL-LDL fractions.

Discussion

Studies on the key enzymes in lipoprotein metabolism, such as lipoprotein lipase in thyroid

disease, are of interest since they may elucidate the mechanisms responsible for the distinct dyslipoproteinemias associated with hypo- and hyperthyroidism. Also, such studies may give important information on the hormonal regulation of the enzymatic activity.

Several models are available for the study of experimental hypothyroidism in the rat. Besides PTU treatment, thyroidectomy, methylmercaptoimidazole administration and ^{131}I treatment have been used. Comparison of data from earlier studies [5,7,8] indicated that there are no consistent qualitative differences with regard to lipase activities, which can be attributed to the different experimental models.

Induction of hypothyroidism in rat, regardless of the method chosen, leads to a marked reduction in food intake and weight gain. In the present study we wanted to differentiate between the effects of hypothyroidism as such and those of the restricted food intake by comparison with pair-fed controls. Also, the short-term effects of food intake was monitored by studying the rats in the fasted as well as the fed state.

Our results on adipose tissue lipoprotein lipase activity are, in general terms, consistent with earlier studies. However, it is evident that the increase in lipoprotein lipase activity is largely due to nutritional status. In contrast to other investigators [5], however, we found consistently higher lipoprotein lipase activities in heart tissue in the hypothyroid rats, with comparatively little influence of food

TABLE IV

PLASMA LIPID AND LIPOPROTEIN CONCENTRATIONS IN HYPOTHYROID RATS AND CONTROL GROUPS

Data are mean $\pm$ S.E. of eight animals, unless otherwise indicated. Significance levels as in Table III. All values are $\text{mmol} \cdot \text{l}^{-1}$.

Group	Triacylglycerol	Cholesterol	HDL cholesterol	HDL phosphatidylcholine
Re-fed				
Hypothyroid, R-PTU	0.79 ± 0.06	$2.46 \pm 0.08^{c,e}$	1.30 ± 0.04	1.29 ± 0.04
Pair-fed, R-PF	0.90 ± 0.09	1.88 ± 0.07^a	1.28 ± 0.02	1.40 ± 0.02
Age-matched, R-AM	1.01 ± 0.08^a	1.69 ± 0.05^c	1.17 ± 0.05	1.20 ± 0.05
Weight-matched, R-WM	0.84 ± 0.09	2.00 ± 0.05	1.31 ± 0.05	1.45 ± 0.03
Fasted				
Hypothyroid, F-PTU	0.85 ± 0.04^c	$2.66 \pm 0.11^{c,e}$	1.34 ± 0.05^a	1.06 ± 0.04
Pair-fed, F-PF	0.98 ± 0.12^a	2.12 ± 0.07	1.38 ± 0.05^b	$1.18 \pm 0.04^a (n = 7)$
Age-matched, F-AM	1.14 ± 0.08^a	1.87 ± 0.10	1.21 ± 0.05	1.15 ± 0.04
Weight-matched, F-WM	1.35 ± 0.07	1.89 ± 0.05	1.14 ± 0.04	1.08 ± 0.02

intake. Further studies are needed to clarify whether this discrepancy may be due to methodological differences (heparin elution of enzyme vs. acetone/diethyl ether preparations).

To elucidate the mechanisms behind the changes in adipose tissue lipoprotein lipase we applied methods designed to reflect different steps in enzyme processing, which includes synthesis of lipoprotein lipase in the fat cell, secretion to the interstitium, binding to the endothelial cells, and catabolism of the enzyme [12]. For practical reasons both retroperitoneal and epididymal adipose tissues were used. Acetone/diethyl ether preparations and heparin eluates both reflect the total fat tissue enzyme activity, although the heparin eluate technique seems to measure predominantly extracellularly located enzyme. Generally, these techniques give qualitatively similar data [13]. In the present study, differences between groups were more readily recorded using the heparin-elution technique, a tendency noted also in studies with human adipose tissue [13]. Lipoprotein lipase secretion from fat cells seems to be a sensitive indicator to hormonal stimulation of enzyme activity [14].

The effects of short-term fasting and age (weight) on adipose tissue lipoprotein lipase activities were in most groups consistent with numerous earlier studies. However, the effect of refeeding was markedly increased in the euthyroid rats on long-term caloric restriction (R-, F-PF), a phenomenon that needs further investigation.

In the fasted rats, the prolonged food restriction in the pair-fed group (F-PF) leads to a decrease in adipocyte lipoprotein lipase activity and lipoprotein lipase secretion rate. Hypothyroidism reverted these changes towards the pattern observed in the weight-matched controls. More pronounced, however, was the increase in lipoprotein lipase activity in whole adipose tissue, especially when measured in heparin eluates. In addition to the effects on intracellular processing, hypothyroidism thus seems to affect enzyme processing extracellularly as well.

In the refed rats, the effect of hypothyroidism as such was much less pronounced and the increased enzyme activities could largely be attributed to long-term food restriction (R-PTU vs. R-PF). However, the moderate increase in the

enzyme activity in heparin eluates of whole tissue was relatively larger than the effects on adipocyte lipoprotein lipase activity and lipoprotein lipase secretion, indicating an effect mainly on the extracellular processing of enzyme.

The nature of the factors affecting intra- and extracellular pools of lipoprotein lipase in hypothyroidism cannot be specified in this study. However, since protein synthesis is low in hypothyroidism [23] it seems unlikely that the increased enzyme activities represent an increased rate of enzyme synthesis. One of the mechanisms involved in lipoprotein lipase regulation in adipose tissue is degradation and inactivation of enzyme occurring before and/or after secretion of enzyme from the fat cell to the interstitial tissue [12]. It is possible that the effects of hypothyroidism represent a decreased rate of degradation intra- and extracellularly. The pronounced differences in lipoprotein lipase activity of the hypothyroid rat and man would, thus, theoretically be due to a different relative sensitivity to protein synthesis inhibition of the enzyme and the degradation systems. Hypothetically, the effects of hypothyroidism on extracellular lipoprotein lipase could also be related to the altered glycosaminoglycan metabolism [24]. For example, an increased number and/or affinity of lipoprotein lipase receptors (heparan sulphate) at the capillary endothelium might contribute to the higher lipoprotein lipase activities in the hypothyroid rat.

The marked species differences in lipoprotein lipase activity in hypothyroidism are also reflected in the changes in plasma lipids and lipoproteins. The elevated lipoprotein lipase activities could explain the lack of hypertriglyceridemia in the hypothyroid rat, whereas the low lipoprotein lipase activities in hypothyroid man are associated with a marked elevation of plasma triacylglycerols [3]. Although comparisons of cholesterol transport in man and the rat should be made with care, the disturbances in cholesterol metabolism appear similar in the two species, and confined mainly to the VLDL-LDL system.

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HEPATIC LIPASE AND LIPOPROTEIN LIPASE ACTIVITIES IN HYPO-
AND HYPERTHYROID RATS DURING FREE AND RESTRICTED FOOD
INTAKE

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ABSTRACT

Experimental hypothyroidism in the rat was accompanied by a decrease of hepatic lipase activities in liver homogenates (7.15 ± 1.10 mU/mg protein, mean $\pm$ SD, vs 13.40 ± 1.63 mU/mg in pair-fed euthyroid animals; $P < 0.005$). Lipoprotein lipase activity of adipose tissue increased (0.94 ± 0.70 vs 0.38 ± 0.13 mU/mg protein in the pair-fed euthyroid rats; $P < 0.01$), whereas the enzyme activity of heart was unaffected. There were no differences in enzyme activities between euthyroid rats eating freely and animals being pair-fed with the hypothyroid group.

In rats treated with triiodothyronine (T_3 , 20 ug/day s.c.) the spontaneous food intake increased. In hyperthyroid rats kept at a standard food intake, the activity of hepatic lipase increased (7.43 ± 1.45 mU/mg protein vs 5.03 ± 1.48 in controls; $P < 0.01$). If hyperphagia was not prevented, hepatic lipase activity increased further (9.01 ± 0.87 mU/mg protein; $P < 0.005$). The activities of lipoprotein lipase in adipose tissue and heart were both significantly decreased in hyperthyroid rats on a standard food intake (0.39 ± 0.08 and 0.32 ± 0.10 mU/mg protein, vs 0.60 ± 0.20 and 0.57 ± 0.19 in euthyroid animals). Spontaneous hyperphagia of T_3 -treated rats did not change the low LPL activity of heart (0.28 ± 0.13 mU/mg protein); however, the decreased enzyme activity of adipose tissue was reversed (0.56 ± 0.09 mU/mg protein).

INTRODUCTION

Alterations in thyroid function are consistently associated with changes in lipid transport and plasma lipoprotein concentrations(1). Both in man and in the rat, the dyslipoproteinemia seems to be partly mediated through alterations in the activities of two enzymes in plasma lipoprotein metabolism, lipoprotein lipase (LPL) and hepatic lipase (HL). In the hypothyroid rat, LPL activity in adipose tissue is increased, while hepatic lipase activity seems to be low (2-5). Reports on the effects of an excess of thyroid hormones on LPL and HL activities are scarce and inconsistent (5,6). Part of these discrepancies may be due to differences in feeding regimen or hormonal treatment, or may be related to the different enzyme sources used, i.e. liver perfusate, postheparin plasma or tissue preparations.

In the present study, we have investigated the effects of hypo- and hyperthyroidism on HL and LPL activities, as measured directly in tissue preparations from rat liver, heart and adipose tissue. Since we have recently demonstrated that the decreased food intake may partly explain the alterations in LPL activity in hypothyroid rats (4), we also designed this study to differentiate between direct and indirect (i.e., via feeding) influences of thyroid hormones on LPL and HL activities.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (ALAB, Stockholm, Sweden) were used. They were maintained in single cages at 23°C on 6 a m - 6 p m light cycle, and were fed R3 chow (EWOS AB, Södertälje, Sweden). Weighing, injections and food administration were done between 4.30 and 5.30 p m, and food was withdrawn on the evening before sacrifice. The animals were killed between 9 and 10 a m by decapitation (first series) or carbon dioxide (second series). Separate control experiments demonstrated no differential effect on LPL or HL activities by these two methods. Blood was obtained by cardiac puncture in the second series. Tissues were immediately removed and frozen.

Experimental design. In the first experimental series, one group of rats received 6-propyl-2-thiouracil (PTU; Sigma Chemical Co.) in their drinking water (0.1 g/l). One group of euthyroid rats were individually pair-fed with those receiving PTU. The animals were maintained on this regimen for 20 days before sacrifice. Another euthyroid group had free access to chow and normal water, and was selected to match the PTU group for body weight at sacrifice.

In the second series, we administered daily subcutaneous injections of 20 ug of 3,5,3'-triiodothyronine (T_3 ; Sigma) to two groups of rats. One of these had free access to chow, whereas in the other food intake was restricted to 20 g daily (12 g at 5.30 p m and 8 g at 8 a m) to prevent hyperphagia. This intake, which is just below the spontaneous food consumption, was chosen in order to facilitate pair-feeding. Euthyroid animals were injected with the solvent (0.01 M NaOH) only, and received 20 g of chow daily. Injections were given during one week, and the last dose was administered 16 h before sacrifice.

Extractions and assays of HL and LPL activities. HL was extracted from liver tissue by a two-step procedure using heparin to solubilize the enzyme (7). About 0.5 g of frozen tissue was homogenized in 5 ml cold Krebs-Ringer HEPES buffer (pH 7.4). One ml of the homogenate was centrifuged for 10 minutes at 4,000xg. The supernate, containing the cytosol fraction and floating fat, was discarded and the pellet was re-suspended in 1 ml cold 0.1 M Tris buffer (pH 9.0) containing heparin (15 U/ml), and kept on ice for 15 minutes. The tube was then recentrifuged and the supernate assayed for HL activity(8) and protein (9). Separate control experiments demonstrated that the two-step procedure increased the yield of HL activity by at least 70% compared to direct homogenization with heparin. Omission of heparin from the second suspension resulted in an 80% decrease in HL activity. The samples could be frozen at -20°C for at least a month without loss of activity.

The LPL activities of epididymal adipose tissue and heart were measured in acetone-ether preparations (10). HL and LPL activities were assayed as previously described (8); dioleoyl phosphatidyl choline (final concen-

Table 1

Body weights, food intake and thyroid hormone concentrations in experimentally hyper- and hypothyroid rats (mean $\pm$ SD; 8 rats/group)

	Experiment I			Experiment II		
	Hypothyroid	Euthyroid pair-fed	Euthyroid ^d ad-lib fed	T ₃ ad lib-fed	T ₃ pair-fed	Euthyroid
Initial weight (g)	224.0 $\pm$ 4.4	225.0 $\pm$ 3.4	-	232.3 $\pm$ 8.3	238.1 $\pm$ 10.0	232.7 $\pm$ 6.4
Weight 1 day before sacrifice (g)	272.1 $\pm$ 4.8	281.3 $\pm$ 9.7	267.3 $\pm$ 4.0	250.5 $\pm$ 9.2	224.9 $\pm$ 9.2	249.4
Food intake 1 day before sacrifice (g/day)	19 $\pm$ 1.4 ^b	19 $\pm$ 1.4 ^b	23.3 $\pm$ 3.8	27.3 $\pm$ 2.9	20	20
Serum:						
T ₃ (nM)	< 0.5 ^c	1.29 $\pm$ 0.11	1.07 $\pm$ 0.12	5.14 $\pm$ 0.90 ^c	5.95 $\pm$ 0.83 ^c	1.48 $\pm$ 0.07
T ₄ (nM)	< 12 ^c	66.0 $\pm$ 6.3	51.0 $\pm$ 6.4	< 12 ^c	< 12 ^c	66.8 $\pm$ 5.3

b = $P < 0.01$, c = $P < 0.005$ compared to euthyroid ad lib-fed rats (Experiment I) or to euthyroid rats (Experiment II), d; n = 6

tration 0.067 mg/ml) was used as substrate emulsifier. The activities were related to protein content (9) of the supernates.

Serum analyses. Concentrations of T_3 and thyroxine (T_4) were determined with radioimmunoassay kits from MILAB, Malmö, Sweden. Kits from Wako Biochemicals, Japan, were used to measure triglycerides and phosphatidyl choline. Serum cholesterol concentrations were determined by chemicals from Boehringer Mannheim GmbH, West Germany. Plasma HDL components were determined after precipitation of apolipoprotein B-containing lipoproteins with dextran and $MgCl_2$ (11).

Statistics. Significance of differences was evaluated by Wilcoxon's method for the unpaired case. For the individually pair-fed groups, Wilcoxon's test for the paired case was used.

RESULTS

Body weights and food intakes are summarized in Table 1. PTU treatment resulted in significant reduction of food intake and weight gain. The T_3 -treated rats increased their spontaneous food intake, but the weight gain was essentially the same as that seen in the controls. If food was restricted, however, the animals lost about 13 g in a week.

Table 1 also shows serum concentrations of thyroid hormones. Both T_3 and T_4 levels were markedly reduced in the PTU-treated rats. T_3 treatment, as expected, resulted in increased serum levels of T_3 . Serum T_4 concentrations were very low, indicating suppression of the endogenous T_4 production.

HL activities in the different groups are shown in Fig 1. Hypothyroidism resulted in a reduction of enzyme activity by about 50 %. HL activities in euthyroid pair-fed rats were not different from those of controls. T_3 treatment led to an increase in HL activity of about 50 % (T_3 , pair-fed). If the rats were allowed to eat freely (T_3 , ad lib-fed) a further increase could be observed.

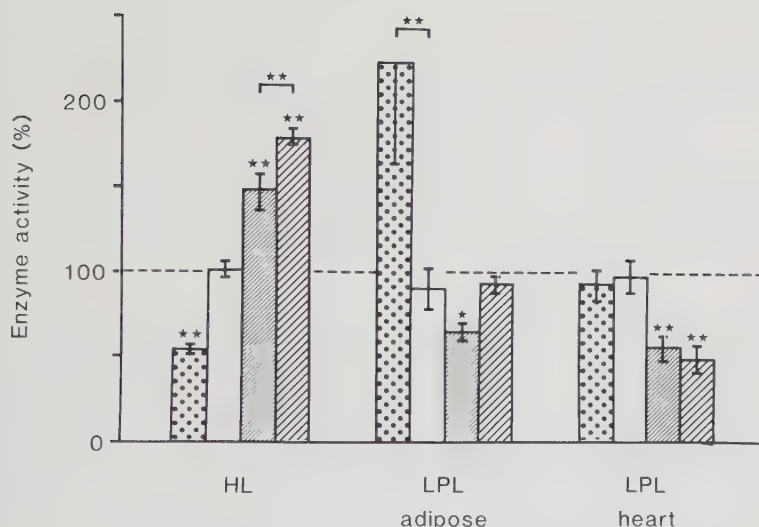


Figure 1

Lipase activities of hypo- and hyperthyroid rats. Data are expressed relative to the mean enzymatic activities of euthyroid animals. Experiment I comprised hypothyroid rats (dotted bars) and individually paired euthyroid animals (open bars). The mean enzyme activities in euthyroid, ad lib-fed rats were (mean $\pm$ S.D.) 13.40 ± 1.61 (HL), 0.42 ± 0.17 (adipose tissue LPL) and 1.20 ± 0.66 (heart LPL) mU/mg protein. Experiment II comprised two groups of rats treated with T_3 : one on a standardized food intake (finely hatched bars) and one eating ad libitum (coarsely hatched bars). Mean enzyme activities in the euthyroid group were 5.03 ± 1.48 (HL), 0.60 ± 0.20 (adipose tissue LPL) and 0.57 ± 0.18 (heart LPL) mU/mg protein. Standard errors of the means are indicated; $n = 8$. * = $P < 0.05$ ** = $P < 0.01$.

LPL activities are demonstrated in Fig 1. Adipose tissue LPL activities were significantly elevated in the hypothyroid rats compared to pair-fed euthyroid controls, while T_3 treatment resulted in a decreased enzyme activity (T_3 , pair-fed). This effect of hyperthyroidism was masked by the spontaneous hyperphagia, since the free-eating hyperthyroid rats had essentially the same enzyme activities as controls.

Heart LPL activity was little affected by hypothyroidism. Hyperthyroid animals had about half the enzyme activities of controls. The pair-fed and the free-eating groups did not differ significantly (Fig 1).

Table 2 shows concentrations of serum lipids. Hypothyroidism led to a decrease in triglyceride levels, which could not be explained by a decreased food intake. HDL cholesterol concentrations were also slightly depressed; total serum cholesterol levels, however, were markedly increased. Experimental hyperthyroidism produced slight decreases in levels of triglyceride and total cholesterol, if hyperphagia was prevented. These changes were absent in the hyperphagic group. An excess of T_3 led to an increase in the concentrations of HDL-phosphatidyl choline, more marked in the hyperphagic animals. In the latter group, HDL-cholesterol was also elevated.

DISCUSSION

Our data demonstrate a regulatory role of thyroid hormones on both HL and LPL activities. The effects of hypo- and hyperthyroidism are partly exerted via changes in feeding (4,12) which have to be taken into account when comparing data from different experiments.

The choice of thyroid hormone for the induction of experimental hyperthyroidism is also of importance. T_3 is generally accepted as the physiologically most active thyroid hormone (13). T_3 is formed mostly by deiodination of T_4 in extrathyroidal tissues (13); the rate of this conversion is influenced by over- and underfeeding as well as by several drugs (13,14). Treatment with T_4 , therefore, may lead to varying degrees of hyperthyroidism due to differences in the rate of T_4 conversion. Indeed, the use of

Table 2

Concentrations of triglycerides (TG), cholesterol and phosphatidylcholine (PC) in rat serum and its HDL subfraction (mean $\pm$ SD).

Concentration (mM)	Experiment I		Experiment II			
	Hypothyroid	Euthyroid pair-fed	Euthyroid ^f ad lib-fed	T ₃ ad lib-fed	T ₃ Pair ^g fed	Euthyroid
TG	0.39 [±] 0.05 ^e	0.50 [±] 0.08	0.67 [±] 0.18	0.72 [±] 0.10	0.58 [±] 0.15	0.70 [±] 0.13
Cholesterol	2.53 [±] 0.22 ^{b,e}	1.85 [±] 0.27	1.72 [±] 0.22	2.15 [±] 0.23	1.81 [±] 0.18	2.15 [±] 0.25
HDL-chole- sterol	1.07 [±] 0.09 ^d	1.24 [±] 0.19	1.04 [±] 0.13 n=4	1.78 [±] 0.21 ^e	1.45 [±] 0.14	1.53 [±] 0.09
HDL-PC	-	-	-	1.83 [±] 0.20 ^{c,d}	1.58 [±] 0.13 ^a	1.29 [±] 0.09
HDL-TG	-	-	-	0.34 [±] 0.07	0.29 [±] 0.05	0.30 [±] 0.04

a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.005$ compared to euthyroid ad lib-fed rats (Experiment I) or to euthyroid rats (Experiment II). d = $p < 0.05$, e = $p < 0.005$ when comparing hypothyroid and euthyroid pair-fed animals (Experiment I) or ad lib-fed and pair-fed T₃-treated animals (Experiment II), f; n = 6

T_4 instead of T_3 may explain some earlier discrepancies in the literature (see below).

HL activities showed increasingly higher values over the full spectrum of thyroid function. The changes in food intake only marginally contributed to these alterations. Our data on hypothyroid rats are consistent with earlier reports (3,5). Fewer data are available concerning HL activity in hyperthyroid rats. Murase and Uchimura (5) could not demonstrate any increased enzyme activity in post-heparin plasma. However, this may be due to their use of T_4 instead of T_3 .

In the preparation of HL from liver homogenates for enzyme assay several problems must be taken into consideration; for example, interference by other lipases in the enzyme preparation (15,16) and the presence of endogenous lipids may affect the specificity and accuracy of the assay. The enzyme activity recorded with our extraction technique fulfills the generally accepted criteria for HL (17): activity at alkaline pH, resistance to 1 M NaCl, and release of enzyme activity from tissue fragments by heparin. The removal of endogenous fat prior to enzyme solubilization greatly increased the enzyme activity measured. The biological variation within the experimental groups was small. However, there was an apparent difference in HL activity between the euthyroid rats of the two experimental series (Fig 1, legend). Since it does not seem to be explained by differences in food intake or by the different methods for sacrifice, we believe it reflects a difference between the two litters used.

The increased LPL activity in adipose tissue of the hypothyroid rat is well documented (2,4,18,19). As all the rats were sacrificed in the fasting state, we did not expect the euthyroid pair-fed animals to differ from the free-eating controls (cf 4). Experimental hyperthyroidism, on the other hand led to a decrease in adipose tissue LPL activity. This response was reversed by spontaneous hyperphagia. Our results agree with those of Shafir & Biale (2), whereas Wilson et al (6) obtained the opposite effect in rats weighing about 220 g. The food intake of the rats is not specified in either study, which makes comparison difficult. Other factors, such as the age of animals and choice of thyroid hormones (6) may probably modify the results.

The previous literature on the regulation of LPL activity in heart by thyroid hormones is divergent: in hypothyroidism, the enzyme activity has been reported to decrease (2) or to increase (4). Similarly, both elevated (2,6,20,21) and lowered (6) enzyme activities have been reported following thyroid hormone administration. The present study demonstrates a consistent decrease in heart LPL activity in hyperthyroidism, while the enzyme activity was not affected by hypothyroidism. Furthermore, the effects of thyroid hormones on heart LPL activity were not mediated via alterations in food intake. The divergent reports may be explained by differences between the experimental animals (age, body weight etc) and/or by differences in experimental design such as PTU dose (20), choice of thyroid hormone (21) or enzyme preparation procedure (20,21). The enzyme activities of this study are expressed per mg of protein whereas other authors have related the activities to tissue wet weight. This may partly explain the apparent discrepancy, as hyperthyroidism causes myocardial hypertrophy (21).

The hypotriglyceridemia of the PTU-treated rats (compared to pair-fed controls) is probably due to the combined effects of the increased adipose tissue LPL activity (cf 4) and a decrease in the hepatic secretion of triglyceride-rich lipoproteins (22).

The increases in HDL-cholesterol and HDL-phosphatidylcholine upon T_3 treatment are consistent with the report of Wilcox et al. (23), who attributed this effect to an increased hepatic secretion of apolipoprotein AI, and thus probably of nascent HDL. Non-HDL-cholesterol was altered in hypo- and hyperthyroidism in a similar way as seen in studies on man (24). Alterations in the receptor-mediated lipoprotein clearance (25) are likely to be the cause.

When comparing data from studies in humans and in rats it is evident that thyroid hormones do not have identical effects on plasma lipoprotein concentrations, and on LPL and HL activities, in the two species. The regulation of HL activity by thyroid hormones seems similar in the two species (cf 24). Non-HDL-cholesterol is also altered in a similar way in humans and rats (1,24). The concentration of this lipoprotein fraction (consisting mainly of IDL and LDL in fasted rats) is regulated to a large extent via

the activity of the lipoprotein receptors; the LDL receptor has been shown to respond to thyroid hormones in both species (25,26).

LPL regulation by thyroid hormones, however, differs considerably between man and the rat. In contrast to the increased or normal enzyme activities recorded in different tissues of the hypothyroid rat, it is well documented that hypothyroid patients have decreased LPL activities of adipose tissue (27,28) and skeletal muscle (28). The increased LPL activities in hypothyroid rats may partly explain the decrease in plasma triglyceride levels. Conversely, the hypertriglyceridemia of overt human hypothyroidism (24) may be partly explained by the decreased enzyme activities.

In humans, there is an inverse relationship between HL activity and plasma levels of HDL-cholesterol under several metabolic conditions including thyroid dysfunction, and HL activity is considered an important regulator of HDL concentrations (24,29). This pattern is not seen in rats; in fact these variables seem directly related in hypo- and hyperthyroidism. Wilcox et al (23) have demonstrated an increased secretion of apolipoprotein AI from perfused livers of T_3 -treated rats. This would indicate an increased secretion of nascent HDL. Such an over-production could well mask a possible relationship between HDL levels and HL activities. However, it cannot be ruled out that the physiological roles of HL are different in the two species.

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THYROID HORMONES STIMULATE THE RELEASE OF HEPATIC LIPASE
FROM CULTURED RAT HEPATOCYTES

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Summary

We have investigated the effects of triiodothyronine (T_3) and thyroxine (T_4) on the heparin-stimulated release of hepatic lipase (HL) activity from cultured rat hepatocytes. Addition of T_4 (1-10 nmol/l) to the culture medium for 24h stimulated HL release from cells derived from normal and hypothyroid rats, whereas T_3 (0.1-10 nmol/l) was active (at the highest concentration) only in hepatocytes from hypothyroid animals. The effects of T_4 could largely be abolished by 5-iodo-2-thiouracil (0.1 mmol/l), an inhibitor of T_4 -5'-deiodinase. This indicates that the effects of T_4 treatment are exerted by T_3 , formed by deiodination in the hepatocytes.

Introduction

Hepatic lipase (HL) is an enzyme involved in the metabolism of plasma lipoproteins, presumably in the interconversion of high density lipoprotein subfractions HDL_2 and HDL_3 (Shirai, Barnhart and Jackson 1981; Nikkilä, Kuusi and Taskinen 1982). The activity of HL is under hormonal influence. Insulin (Knauer et al. 1982), gestagens (Ehnholm et al. 1975) and thyroid hormones (Valdemarsson et al. 1983) increase its activity in tissue homogenates or post-heparin plasma. Present data indicate that HL is synthesized in the hepatocytes, and is then transported to the capillary endothelium, its physiological site of action (Cryer, 1983). It is released from the endothelial cells by heparin, and may be quantitated in postheparin plasma. Theoretically, thyroid hormones may regulate HL activity via effects on enzyme synthesis in the hepatocytes, on enzyme transport to the endothelial cells, or by influencing the binding of the enzyme at the vascular endothelium. In this study, we have investigated the effects of thyroxine and triiodothyronine on the heparin-stimulated release of HL from isolated rat hepatocytes in culture. We also wanted to compare the effects of triiodothyronine and thyroxine in this system.

Materials and Methods

Male Sprague-Dawley rats weighing about 250 g (ALAB, Stockholm) were used. In some animals, hypothyroidism was induced by 6-propyl-2-thiouracil (PTU), added to the drinking water in a concentration of 0.1 g/l.

Hepatocytes were prepared by collagenase perfusion via the portal vein in ether-anesthetized animals (Berry and Friend 1969; Florén and Nilsson 1978) using the conditions of Seglen (1973). The perfusate was buffered with 40 mmol/l HEPES. The hepatocytes were cultured in primary monolayers (Bonney et al 1974; Bisell, Hammaker and Meyer 1975; Lin and Snodgrass 1975). The culture medium was Leibowitz L-15 (pH 7.4) containing 5% fetal calf serum, 28 mmol/l HEPES, 1 mmol/l sodium succinate, 100 ug/ml penicillin G and 50 ug/ml gentamicin. Collagen-coated 60 mm petri dishes (Falcon 1007) were used (Lin and Snodgrass 1975). About 3.5×10^6 suspended cells were plated on each dish in a volume of 2.5 ml. The dishes were placed in humidified air at 37°C. After 24 h, the cells had arranged in monolayers of trabecular cell aggregates. We then removed the medium and added 2.5 ml of serum-free medium, with 50 ul of triiodothyronine (T_3) or of thyroxine (T_4), dissolved in 0.01 mol/l NaOH. Final hormone concentrations were 1 and 10 nmol/l (T_4) and 0.1, 1, and 10 nmol/l (T_3). No appreciable change of pH followed the addition of hormone. After a further 24h incubation at 37°C, the medium, together with non-adherent cells, was removed and discarded. One ml of serum-free medium, containing the same hormone concentrations and 100 U of heparin, was added to each dish. After 2 h, the medium was removed and frozen for later assay of HL activity. The cells were scraped off with a rubber policeman. Protein content of the cells was determined by the method of Lowry(1951). Incubations were also done in the presence of 0.1 mmol/l 5-iodo-2-thiouracil (ITU). This compound is a potent inhibitor of hepatic 5'-deiodinase, which catalyzes the conversion of T_4 to T_3 . (Chopra et al. 1982). All incubations were performed in triplicate together with quadruple hormone-free control incubations. Statistical evaluation was done by the Wilcoxon test for the paired case.

Fetal calf serum was included in the first incubation to improve attachment of cells, but was excluded due to its T_4 content (final concentration about 15 nmol/l), during the second incubation. The concentration of heparin, and the time allowed for release of HL activity, were chosen after preliminary experiments.

HL activity was assayed essentially as previously described (Nilsson-Ehle and Ekman 1977), using dioleoyl phosphatidyl choline as substrate emul-

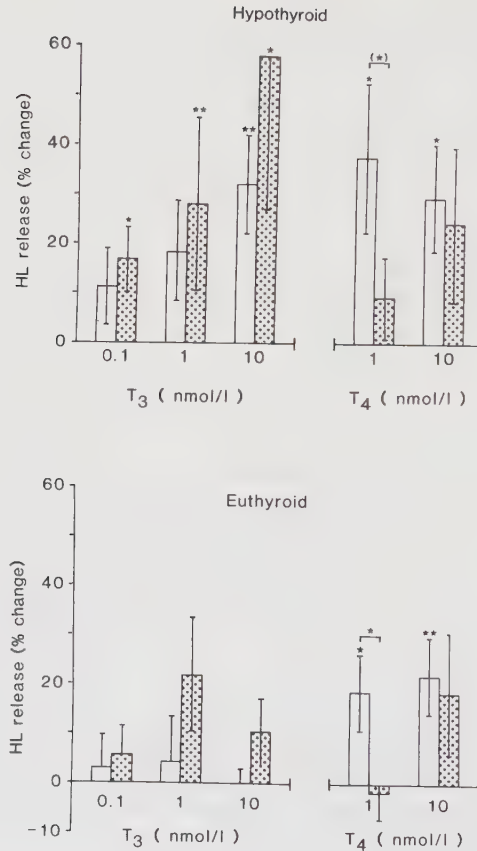


Figure 1

Relative change ($\pm$ SEM) in heparin-stimulated release of hepatic lipase from hepatocytes from hypothyroid (upper panel) and euthyroid (lower panel) rats incubated with increasing concentrations of thyroid hormones. Dotted bars indicate the presence of ITU. Each panel represents nine experiments in four rats. HL activities in hormone-free control incubations were 0.073 ± 0.032 (hypothyroid), 0.066 ± 0.030 (hypothyroid; ITU present), 0.058 ± 0.012 (euthyroid), and 0.059 ± 0.014 (euthyroid; ITU present) mU/mg protein. (mean $\pm$ SD).

sifier. To avoid dilution of the samples the incubations were made without addition of 2 mol/l NaCl; this did not affect the enzyme activity measured.

Leibowitz L-15 medium, fetal calf serum, and HEPES (N-2-hydroxyethylpiperazine-N' - 2-ethanesulphonic acid) were purchased from Flow Laboratories, Irvine, Ayrshire, U.K. Acid-soluble calf skin collagen, dioleoyl phosphatidyl choline, T_3 and T_4 were from Sigma Chemical Co, St Louis, MO, USA. Collagenase (CLS 143-180 units/mg) was from Millipore Corp., Freehold, NJ, USA. Preservative-free heparin was from KABI Vitrum, Stockholm, Sweden. ITU was bought from ICN Nutritional Biochemicals, Plainview, NY, USA. Radioimmunoassay kits from MILAB, Malmö, Sweden, were used to measure serum contents of T_3 and T_4 .

Results and Discussion

Serum levels of T_3 and of T_4 were markedly reduced in the hypothyroid rats (data not shown). In cells from hypothyroid rats, HL release was stimulated by T_3 (10 nmol/l) and by T_4 (1 and 10 nmol/l) (Fig 1). The effect of T_4 could be reproduced in cells from euthyroid animals; however, in such cells, T_3 was inactive even at the highest concentration. The apparent increase in hepatocyte sensitivity to T_3 caused by hypothyroidism may have several explanations. Up-regulation of nuclear hormone receptor may occur but has not been clearly demonstrated (Oppenheimer et al. 1982). Alterations in the kinetics of hormone internalization into hepatocytes (Krenning et al. 1982) could also contribute to the differences observed between hepatocytes from hypo- and euthyroid rats.

The presence of ITU did not impair the T_3 stimulation of enzyme release; rather, a slight increase in enzyme activity could be seen. In contrast, the stimulatory effect of T_4 on HL release could largely be abolished by ITU, indicating that T_4 by itself is not active, but has to be converted to T_3 . The inhibitory effect of ITU was most pronounced at a T_4 concentration of 1 nmol/l (Fig 1).

The moderate, non-significant increase of HL release in incubations with 10 nmol/l T_4 and ITU may have several explanations. Since ITU is a non-compet-

titive inhibitor of T_4 conversion (Chopra et al. 1982) it is unlikely that insufficient inhibition of conversion is the cause. Rather, we believe that these data reflect a weak, endogenous activity of T_4 . It should be stressed that hormone-binding proteins are absent from our incubation system. A free T_4 concentration of 10 nmol/l is well above the physiological range.

In conclusion, this study demonstrates that the stimulatory effect of thyroid hormones is at least partly exerted via effects on the hepatocytes proper. Further experiments are needed to differentiate between effects on various steps in cellular handling of the enzyme. Our data also imply that T_3 , rather than T_4 is the most active iodothyronine, in agreement with data concerning other hepatic enzymes (Oppenheimer et al. 1982).

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MICROSURGICAL DENERVATION OF RAT ADIPOSE TISSUE:

LACK OF EFFECT ON LIPOPROTEIN LIPASE

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Received September 28, 1981

SUMMARY: The effects of unilateral microsurgical denervation on the activity of lipoprotein lipase in rat epididymal adipose tissue have been investigated. This technique provides successful denervation in 30-70% of the operations. Lipoprotein lipase activities were not different from those on the non-operated side, although the concentrations of norepinephrine were at least ten times lower. It is concluded that lipoprotein lipase of rat adipose tissue is unlikely to be regulated by an adrenergic nervous mechanism.

Lipoprotein lipase (EC 3.1.1.34) catalyzes the key reaction in the degradation of triglyceride rich lipoproteins, i.e. VLDL and chylomicrons. The activity of the enzyme varies considerably with the metabolic state of the organism (1). It is generally agreed (2) that the enzyme is mainly regulated by hormones like insulin and catecholamines (3). However, little is known about a possible nervous regulation of the enzyme. In adipose tissue, the hormone-sensitive lipase, which catalyzes the hydrolysis of stored triglycerides, is known to be regulated by an adrenergic nervous mechanism (4). The purpose of this investigation was to study if adrenergic denervation has any effect on lipoprotein lipase activity in rat adipose tissue.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 150-200 g were used. Larger rats were unsuitable because of larger accumulation of retroperitoneal fat. Under ether anesthesia, the abdominal wall was opened in the middle and the intestines were displaced to the left in a moist cloth. The spermatic vessels on the right side were exposed. Using an operating microscope (Zeiss OpMi-6) and microsurgical technique the vessels were freed from surrounding structures. The nerves, running close to the vessels, were freed with utmost precaution, in order to avoid injury to the spermatic artery or vein, and to minimize disturbance in the blood-flow of the vessels. When dissected free, about 5 mm of the nerves were removed. We found it easier to identify the nerves, if the operating area was dyed by 2 - 3 drops of 1% toluidine blue. A magnification of at least 40 times was necessary for successful operations. After denervation, the intestines were replaced, and the peritoneal cavity closed.

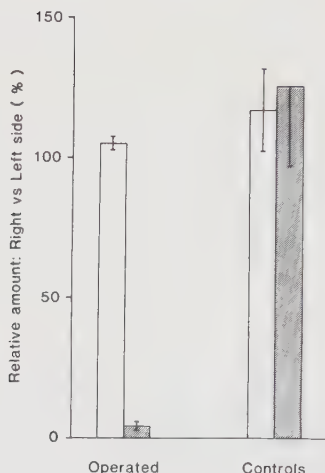


FIG. 1 Lipoprotein lipase activities (open symbols) and norepinephrine contents (cross-hatched symbols) of fat pads from non-operated control rats (n=11) and rats successfully denervated on the right side (n=5). Bars indicate S.E.M.

Five to nine days after the operation the rats were killed by stunning between 9.30 and 10.30 a.m.. The epididymal fat pads were removed and assayed for lipoprotein lipase activity and norepinephrine content. As a consequence of the unilateral operation, the fat of the non-operated side served as control for each individual rat. Non-operated control rats were killed and the two fat pads processed the same way.

Lipoprotein lipase activity was determined as follows: About 100 mg of adipose tissue were incubated for 20 minutes in 0.5 ml of Krebs-Ringer- HCO_3^- buffer (pH 7.4) containing human serum (10%, v/v), glucose (5.6 mM), insulin (0.1 U/ml) and heparin (6 U/ml). After the incubation, the buffer was removed and assayed for lipoprotein lipase activity using [^3H]-tri-oleylglycerol as substrate (5). Phosphatidylcholine (final conc. 0.067 mg/ml) was used instead of lysophosphatidylcholine as substrate emulsifier. The fat of the remaining tissue was extracted by isopropanol: heptane: 0.5 M H_2SO_4 (40:10:1; v/v/v). After partition with an acidic aqueous phase, the upper phase was separated and dried under nitrogen to allow determination of the lipid weight of the sample. Enzymatic activity was expressed as mU/g lipid; one mU represents the release of 1 nmol fatty acid per min.

Norepinephrine content was determined essentially according to da Trada & Zürchner (6). In short, catecholamines were extracted by perchloric acid and methylated using catechol-O-methyl-transferase with [^3H]-S-adenosyl-methionine as methyl group donor. The resulting labelled compounds were separated by thin layer chromatography and quantitated by liquid scintillation counting. The content was expressed in ng/mg wet weight.

The denervation was considered successful, if the norepinephrine content of the operated fat pad was less than 10% of that of the contralateral fat pad. Furthermore, it was ascertained by light microscopy that the removed specimens consisted of nerve tissue.

Table 1 Lipoprotein lipase (LPL) activity and norepinephrine (NE) content of epididymal adipose tissue from five successfully denervated rats.

Rat	Nutritional status	LPL activity (mU/g lipid)		NE content (ng/mg wet weight)	
		Op side	Non-op side	Op side	Non-op side
1	fed	372	341	0.001	0.083
2	fed	228	214	not detectable	0.061
3	fasted overnight	6.0	5.4	0.002	0.043
4	fasted overnight	3.7	3.6	0.009	0.098
5	fasted overnight	2.1	2.2	0.002	0.036

RESULTS AND DISCUSSION

The operations were successful in 30-70% (data not shown). It is thus possible to remove perivascular adrenergic nerves from blood vessels with a diameter of 0.3 - 0.5 mm with a reasonable rate of success. The technique is readily applicable to similar research problems, also in tissues other than fat.

The norepinephrine content of successfully denervated adipose tissue was 0 - 9.2% of that of the contralateral fat pad (Table I, fig. 1). Lipoprotein lipase activities, however, did not differ significantly (Table I). As expected, there were no significant differences in enzyme activity or norepinephrine content between the fat pads of the control rats (fig. 1). The lack of change in lipoprotein lipase activity despite successful denervation indicates that lipoprotein lipase is unlikely to be regulated by an adrenergic nervous mechanism. However, it should be remembered that the rat differs from man in several aspects of its lipid metabolism. One difference, which may be relevant in this context, is the fact that human white adipocytes contain α_2 - adrenergic receptors which mediate a reversal of lipolysis (4,7). Such receptors seem to be absent, or sparse, in rat adipocytes (4,7).

ACKNOWLEDGEMENTS

We are grateful to dr Anders Björklund for the norepinephrine determinations, and to dr Mats Ekelund for microscopy of tissue specimens.

Ms Gerd Nilsson and Ms Kerstin Fogelström gave excellent technical assistance.

The help of Monica Radnell, at the Department of Experimental Surgery, is also gratefully acknowledged.

This project was sponsored by the Swedish Medical Research Council (Grant 04966), the Medical Faculty of the University, the Pahlsson foundation and Anna-Lisa & Sven-Eric Lundgrens' foundation for medical research.

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HEPATIC LIPASE ACTIVITY INCREASES AFTER LIVER DENERVATION
IN THE RAT

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SUMMARY

We have investigated the effects of hilar denervation of rat liver upon the activity of hepatic lipase determined in tissue extracts. Denervated animals had an enzyme activity of 7.89 ± 0.37 mU/mg protein, compared to 6.45 ± 0.43 in sham-operated controls (mean $\pm$ standard error; $p = 0.05$). Food intake was not altered by denervation. We conclude that hepatic innervation may contribute to the regulation of hepatic lipase activity.

INTRODUCTION

Hepatic lipase (EC 3.1.1.3) (HL) is an enzyme secreted by hepatocytes which is catalytically active on the endothelial surface of the hepatic capillaries (Nilsson-Ehle, Garfinkel & Schotz 1980). Its role in the metabolism of plasma lipoproteins is still controversial; however, several studies suggest that HL is involved in the metabolism of high density lipoprotein (HDL), by catalyzing the conversion of the HDL₂ to the HDL₃ subfraction (Shirai, Barnhart and Jackson 1981).

The activity of HL is under metabolic control. Insulin (Elkeles & Hambley 1976, Knauer et al 1982) and gestagens (Ehnholm et al 1975, Glueck et al 1976) are the best known stimulators of enzyme activity; we have recently demonstrated that thyroid hormones increase HL activity in man (Hansson, Valdemarsson & Nilsson-Ehle 1983) and the rat (to be published). The possible role of nervous influence on the regulation of HL has not been studied before. The present study demonstrates that hepatic denervation increases HL activity.

MATERIALS AND METHODS

Experimental procedure. Male Sprague-Dawley rats, weighing about 220 g, were used. They were housed in single cages on a 6 a m - 6 p m light cycle at 23°C. Since some authors (Tordoff, Novin and Russek 1982; but cf Louis-Sylvestre et al 1980)

suggest an effect on feeding by denervation, two sham-operated control groups were used: one group was individually pair-fed to the denervated animals, the other had free access to food. Each group contained ten animals. The animals were fed Ewos R3 chow (Ewos AB, Södertälje, Sweden); pair-fed animals were given chow at 5 p m.

Hilar denervation of the liver was performed by microsurgical technique (Holmin et al, in press). Without previous fasting the animals were given light ether anaesthesia. The abdomen was opened with a midline incision, the gut exteriorized to the left and wrapped in a cloth moistened with saline. The porta hepatis was visualized using a Zeiss OPMI 6-S operating microscope. The peritoneum over the proximal part of the hepatoduodenal ligament was incised close to the porta hepatis. This area lies superior to the most cranial part of the pancreas and well away from the gastroduodenal artery and contains both the anterior and posterior plexus of the liver nerves. In the small opening of the ligament 2-3 drops of 1% toluidine blue solution was instilled, to improve visualization of the nerves. Superfluous dye was wiped off after three minutes. There were generally two major nerves around the hepatic artery, and single nerves were also seen close to the portal vein, along the bile duct and in the hepatoduodenal ligament. The nerves were carefully cut, and 2-3 mm removed. Care was taken to spare the vagus branches. The abdomen was closed by double layers of sutures. The control groups were sham operated using the same procedure, including the dye, but without cutting the nerves.

After the surgical procedure the animals were kept for one week. Food intakes and body weights were recorded daily. The animals were then fasted overnight with free access to water, and were sacrificed by CO₂ suffocation between 9 and 10 a.m. The thoracic cavity was immediately opened and blood was drawn by cardiac puncture. About 9 ml could be withdrawn; bleaching of the liver was observed. A piece of the left liver lobe (roughly 3 g), extending 1 cm into the parenchyma, was removed and frozen at -70°C, for later assay of HL activity. Separate control experiments demonstrated that tissue enzyme activity

was stable for at least one month. A piece of the adjacent tissue was taken for norepinephrine determination.

Measurements of HL activity. Preparation of HL from liver homogenates was performed by a modification of Assmann's method (Assmann et al 1973). About 500 mg of frozen tissue were homogenized in 5 ml cold Krebs-Ringer-HEPES-buffer (pH 7.4). One ml of the suspension was centrifuged at 4,000~~x~~g for 5 minutes at 6°C. The supernate, containing the floating fat and cytosol fractions, was discarded and 1 ml of cold 0.1M Tris buffer (pH 9), containing 15 IU of heparin, was added to the microsomal fraction. After suspension and incubation for 15 min in an ice-bath, the suspension was centrifuged as above and the supernate removed for assay. It could be stored at -20°C for several months. The first homogenization was introduced to remove any triglyceride droplets, to which the enzyme could adsorb. This step increased the yield by about 70% compared to direct homogenization with the heparin-containing buffer. The data reported in this paper refer to the heparin-released lipase activities found in the second supernate.

HL activity was assayed by the method of Nilsson-Ehle & Ekman (1977), using dioleoyl-phosphatidylcholine (final concentration 0.067 mg/ml) as substrate emulsifier. The enzymatic activity was related to the protein content of the sample (Lowry et al 1951).

Other analyses. Tissue contents of norepinephrine were determined by the method of Eriksson & Persson (1982). Serum concentrations of glucose and bilirubin, and activities of alanine and aspartate aminotransferases, alkaline phosphatase and gamma-glutamyl transpeptidase were determined by standard laboratory procedures in a Greiner GSA II automatic analyzer.

TABLE I Body weights and food intakes of the three groups.

	Body weight (g) two days post- operatively	Food intake (g/day)	Body weight (g) before sacri- fice	Food intake (g/day)
I Denervated	223.1 $\pm$ 2.8 ^a	21.3 $\pm$ 1.0	255.3 $\pm$ 3.9	24.8 $\pm$ 0.8
II Sham operated pair-fed	229.1 $\pm$ 3.8	21.3 $\pm$ 1.0	249.3 $\pm$ 3.5 ^b	24.8 $\pm$ 0.8
III Sham-operated free access to food	217.3 $\pm$ 3.6	20.4 $\pm$ 3.3	252.6 $\pm$ 4.1	24.0 $\pm$ 1.0

a) Mean $\pm$ SD, n = 9 in each group.

b) For practical reasons, the pair-fed rats were sacrificed one day earlier than the others. The weights of this group are thus not fully comparable.

RESULTS

Body weights and food intakes are shown in Table I. They were nearly identical in the three groups. Glucose and bilirubin concentrations as well as activities of aminotransferases, alkaline phosphatase and glutamyltranspeptidase were also unaffected by denervation.

Hilar denervation resulted in a reduction of about 80% in the liver content of norepinephrine (Table II). One denervated rat was excluded from the study, since its tissue content of norepinephrine (146 ng/g) indicated an unsuccessful denervation. One rat in each of the sham-operated groups died postoperatively. Each group thus consists of nine animals.

HL activities were about 20% higher in the denervated rats compared to the sham-operated ones. This difference was significant ($p = 0.05$) between denervated and controls with free access to food (Wilcoxon's rank sum test). The same level of significance applied when the denervated group was compared to the combined control groups (Table II).

DISCUSSION

The HL activities measured in liver tissue showed a small biological variation compared to e.g. lipoprotein lipase in various tissues (Hansson, Nordin & Nilsson-Ehle 1983). The method described here is simple and reproducible, and recovery of enzyme activity is similar to that described by others (Cordle, Yeaman & Clegg 1983).

The present surgical procedure is not designed to produce complete denervation, but norepinephrine content is substantially decreased. The denervation is produced with less interference with hepatic circulation, than is needed for autotransplantation and other denervation techniques. Since the vagus branches are spared, and since peptidergic nerves have not been identified in the resected nerve specimens (Holmin et al. in press), one can

TABLE II Effects of hilar denervation on liver contents of norepinephrine and HL activity^a

	Norepinephrine (ng/g wet weight)	HL activity (mU/mg protein)
I Denervated	23.4 $\pm$ 6.1 ^c	7.89 $\pm$ 0.37 ^b
II Sham-operated pair-fed	144.1 $\pm$ 26.2	6.64 $\pm$ 0.51
III Sham-operated free access to food	142.4 $\pm$ 27.1	6.45 $\pm$ 0.43

a = Mean $\pm$ Standard error, n = 9 in each group

b = p < 0.05 compared to III and to II+III

c = p < 0.001 compared to II and III

assume that this denervation procedure involves mainly the adrenergic nerves. Our results, demonstrating that hilar denervation clearly increases HL activity, would thus indicate that adrenergic influence could decrease the enzyme activity. Although this function of hepatic nerves has not been reported previously, neural regulation of hepatic metabolism has been documented in other metabolic pathways such as glycogenolysis and glycogenesis (Lautt 1980, Hartmann, Beckh & Jungermann 1982). An effect on cholesterol metabolism has also been described (Shanygina et al. 1981).

The observed increase in enzyme activity may represent a direct effect of the denervation on enzyme processing, or it may be secondary to other alterations in hepatic metabolism and physiology. For example, liver hemodynamics is markedly altered after denervation (Kullendorff et al, in press). Another possibility is that the change in HL activity occurs as a consequence of alterations in carbohydrate metabolism after denervation.

Considerable quantitative (Metz & Forssmann 1980, Moghimzadeh, Nobin & Rosengren 1980) and qualitative (Lautt, 1980) species differences exist in hepatic innervation, which makes extrapolation of the present data to human metabolism difficult. As the liver nerves are often unintentionally injured at liver resections, studies in humans are at least theoretically feasible.

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